FORM PTO-1390 (REV. 1-98) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY 'S DOCKET NUMBER: **WALLACH=21**

U.S. APPLICATION NO. (If known, see 37 CFR 1.5): 09/155676

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/IL97/00117	**01 April 1997**	**02 April 1996**

TITLE OF INVENTION
MODULATORS OF TNF RECEPTOR ASSOCIATED FACTOR (TRAF), THEIR PREPARATION AND USE

APPLICANT(S) FOR DO/EO/US
David WALLACH et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Aplication under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
    ☒ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:

    1. **A courtesy copy of the specification as originally filed.**
    2. **A courtesy copy of the first page of the International Publication (WO97/37016).**
    3. **A courtesy copy of the International Search Report.**
    4. **A courtesy copy of the International Preliminary Examination Report.**
    5. **Formal drawings, 76 sheets, figures 1-7.**

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
	PCT/IL97/00117	WALLACH=21

17. ☒ The following fees are submitted:

	CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $1070.00		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $930.00		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO $790.00		
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) $720.00		
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) $98.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 930.00	
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	50 - 20 =	30	x $22.00	$	
Independent claims	6 - 3 =	3	x $82.00	$	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $270.00	$	

TOTAL OF ABOVE CALCULATIONS =	$ 930.00	
Reduction of 1/2 for filing by small entity, if applicable. A Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28). +	$	
SUBTOTAL =	$ 930.00	
Processing fee of $130.00 for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)).	$	
TOTAL NATIONAL FEE =	$ 930.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +	$	
TOTAL FEES ENCLOSED =	$ 930.00	
	Amount to be refunded:	$
	charged:	$

a. ☒ A check in the amount of $ 930.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-4035 . A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

BROWDY AND NEIMARK, P.L.L.C.
419 Seventh Street N.W., Suite 300
Washington, D.C. 20004

SIGNATURE

Roger L. Browdy
NAME

25,618
REGISTRATION NUMBER

Date of this submission: October 2, 1998

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	Art Unit:
David WALLACH et al.	)	
IA No.: PCT/IL97/00117	)	
IA Filed: 01 April 1997	)	Washington, D.C.
U.S. App. No.: (Not Yet Assigned)	)	
National Filing Date: (Not Yet Received)	)	October 2, 1998
For: MODULATORS OF TNF...	)	Docket No.: WALLACH=21

PRELIMINARY AMENDMENT

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Contemporaneous with the filing of this case and prior to calculation of the filing fee, kindly amend as follows:

IN THE CLAIMS

Claim 4, line 1, delete "any one of claims 1 to 3", and insert therefor --claim 1--.

Claim 5, line 1, delete "any one of claims 1 to 4", and insert therefor --claim 1--.

Claim 6, line 1, delete "any one of claims 1 to 4", and insert therefor --claim 1--.

Claim 8, line 1, delete "or 6".

Claim 11, line 1, delete "or 10".

Claim 13, line 1, delete "any one of claims 1-12", and insert therefor --claim 1--.

Claim 16, line 2, delete "any one of claims 13-15", and insert therefor --claim 13--.

Claim 17, line 2, delete "any one of claims 1-12", and insert therefor --claim 1--.

Claim 23, line 4, delete "any one of claims 17-20", and insert therefor --claim 17--.

Claim 27, line 4, delete "any one of claims 1-11", and insert therefor --claim 1--.

Claim 29, line 4, delete "any one of claims 17-20", and insert therefor --claim 17--.

Claim 30, lines 1 and 2, delete "any one of claims 17-20", and insert therefor --claim 17--.

Claim 31, line 1, delete "any one of claims 23-30", and insert therefor --claim 23--.

Claim 32, line 3, delete "any one of claims 17-20", and insert therefor --claim 17--.

Claim 33, lines 4 and 5, delete "any one of claims 17-20", and insert therefor --claim 17--.

Claim 34, lines 3 and 4, delete "any one of claims 1-11", and insert therefor --claim 1--.

Claim 35, line 3, delete "any one of claims 17-20", and insert therefor --claim 17--.

Claim 36, line 3, delete "any one of claims 17-20", and insert therefor --claim 17--.

Claim 39, line 4, delete "or 20".

Claim 40, line 3, delete "any one of claims 17-20", and insert therefor --claim 17--;

lines 5 and 6, delete "any one of claims 17-20", and insert therefor --claim 17--;

line 8, delete "any one of claims 17-20", and insert therefor --claim 17--; and

line 10, delete "any one of claims 17-20", and insert therefor --claim 17--.

Claim 43, line 2, delete "any one of claims 17-20", and insert therefor --claim 17--.

Claim 44, line 2, delete "any one of claims 17-20", and insert therefor --claim 17--.

Claim 46, line 2, delete "any one of claims 17-20", and insert therefor --claim 17--.

Claim 49, lines 2 and 3, delete "any one of claims 17-20", and insert therefor --claim 17--; and

line 5, delete "any one of claims 17-20", and insert therefor --claim 17--.

REMARKS

The above amendments to the claims are being made in order to eliminate any properly multiply dependent claims, for the purpose of reducing the filing fee. Please enter this amendment prior to calculation of the filing fee in this case.

Favorable consideration and allowance are earnestly solicited.

Respectfully submitted,

BROWDY AND NEIMARK, P.L.L.C.
Attorneys for Applicants

By _____
Roger L. Browdy
Registration No. 25,618

RLB:bcs
f:\user3\98oct\wal21.pa

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	Art Unit:
David WALLACH et al.	)	
IA No.: PCT/IL97/00117	)	
IA Filed: 01 April 1997	)	Washington, D.C.
U.S. App. No.: (Not Yet Assigned)	)	
National Filing Date: (Not Yet Received)	)	October 2, 1998
For: MODULATORS OF TNF...	)	Docket No.: WALLACH=21

SUPPLEMENTAL PRELIMINARY AMENDMENT

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Prior to examination on the merits, kindly amend as follows:

IN THE CLAIMS

Please amend the claims as follows:

19 (amended). A TRAF-binding protein, isoforms, fragments, analogs and derivatives thereof [, according to claim 17 being the] encoded by a DNA sequence according to claim 1, said protein, isoforms, fragments, analogs and derivatives thereof being capable of binding to at least the portion or the TRAF2 protein between amino acids 222-501 of TRAF2, wherein said protein is NIK protein, isoforms, analogs, fragments [and] or derivatives thereof [encoded by the DNA sequence according to any one of claims 1-12].

Claim 21, line 1, after "producing a", insert -- TRAF-binding --; and

line 2, delete "according to any one of claims 17-19".

Claim 22, lines 2-4, delete "or 18;" to the end of the claim.

Claim 25, line 7 and 8, delete "according to any one of claims 17-20".

28 (amended). A method according to claim 27 wherein said oligonucleotide sequence is introduced to said cells via a recombinant animal virus vector carrying a sequence encoding a viral surface protein (ligand) that is capable of binding to a specific cell surface receptor on the surface of said cells to be treated and a [of claim 25 wherein said] second sequence [of said virus encodes] encoding said oligonucleotide sequence.

Please add the following new claim:

50. Antibodies or active fragments or derivatives thereof, specific for the NIK protein, isoform, analog, fragment or derivative thereof according to claim 19.

REMARKS

Claims 1-50 presently appear in this case. The present amendments are being made in order to correct the improper

multiply dependent claims and to place them in singly dependent form.

Favorable consideration and allowance are earnestly solicited.

Respectfully submitted,

BROWDY AND NEIMARK, P.L.L.C.
Attorneys for Applicants

By _____
Roger L. Browdy
Registration No. 25,618

RLB:bcs
Telephone No.: (202) 628-5197
Facsimile No.: (202) 737-3528
f:\user3\9&oct\wal21.spa

MODULATORS OF TNF RECEPTOR ASSOCIATED FACTOR (TRAF), THEIR PREPARATION AND USE

Field of the Invention

The present invention concerns DNA sequences encoding proteins capable of binding to TRAF2, the proteins encoded thereby, and the use of said proteins and DNA sequences in the treatment or prevention of a pathological condition associated with NF-κB induction or with any other activity mediated by TRAF2 or by other molecules to which said proteins bind.

Background of the Invention

The Tumor Necrosis Factor/Nerve Growth Factor (TNF/NGF) receptor superfamily is defined by structural homology between the extracellular domains of its members (Bazan, 1993; Beutler and van Huffel, 1994; Smith et al., 1994). Except for two receptors, the p55 TNF receptor and Fas/APO1, the various members of this receptor family do not exhibit clear similarity of structure in their intracellular domains. Nevertheless, there is much similarity of function between the receptors, indicating that they share common signaling pathways. One example for this similarity is the ability of several receptors of the TNF/NGF family to activate the transcription factor NF-κB. This common ability was ascribed to a capability of a cytoplasmic protein that activates NF-κB, TNF Receptor Associated Factor 2 (TRAF2) to bind to the structurally-dissimilar intracellular domains of several of the receptors of the TNF/NGF family. By what mechanisms does TRAF2 act and how is its responsiveness to the different receptors to which it binds coordinated is not known.

TRAF2 is a member of a recently described family of proteins called TRAF that includes several proteins identified as, for example, TRAF1, TRAF2 (Rothe, M., Wong, s.c., Henzel, W.J. and Goeddel, D (1994) Cell 78:681-692; PCT published application WO 95/33051), TRAF3 (Cheng, G. et al. (1995)), and TRAF6 (see Cao et al., 1996a).

All proteins belonging to the TRAF family share high degree of amino acid identity in their C-terminal domains, while their N-terminal domains may be unrelated. As shown in a schematic illustration of TRAF2 (Fig. 1), the molecule contains a ring finger motif and two TFIIIA-like zinc finger motifs at its C-terminal area. The C-terminal half of the molecule

includes a region known as the "TRAF domain" containing a potential leucine zipper-region extending between amino acids 264 - 358 (called N-TRAF), and another part towards the carboxy end of the domain between amino acids 359 - 501 (called C-TRAF) which is responsible for TRAF binding to the receptors and to other TRAF molecules to form homo- or heterodimers.

Activation of the transcription factor NF-κB is one manifestation of the signaling cascade initiated by some of the TNF/NGF receptors and mediated by TRAF2. NF-κB comprises members of a family of dimer-forming proteins with homology to the Rel oncogene which, in their dimeric form, act as transcription factors. These factors are ubiquitous and participate in regulation of the expression of multiple genes. Although initially identified as a factor that is constitutively present in B cells at the stage of Igκ light chain expression, NF-κB is known primarily for its action as an inducible transcriptional activator. In most known cases NF-κB behaves as a primary factor, namely the induction of its activity is by activation of pre-existing molecules present in the cell in their inactive form, rather than its de-novo synthesis which in turn relies on inducible transcription factors that turn-on the NF-κB gene. The effects of NF-κB are highly pleiotropic. Most of these numerous effects share the common features of being quickly induced in response to an extracellular stimulus. The majority of the NF-κB-activating agents are inducers of immune defense, including components of viruses and bacteria, cytokines that regulate immune response, UV light and others. Accordingly, many of the genes regulated by NF-κB contribute to immune defense (see Blank et al., 1992; Grilli et al., 1993; Baeuerle and Henkel, 1994, for reviews).

One major feature of NF-κB-regulation is that this factor can exist in a cytoplasmic non-DNA binding form which can be induced to translocate to the nucleus, bind DNA and activate transcription. This dual form of the NF-κB proteins is regulated by I-κB - a family of proteins that contain repeats of a domain that has initially been discerned in the erythrocyte protein ankyrin (Gilmore and Morin, 1993). In the unstimulated form, the NF-κB dimer occurs in association with an I-κB molecule which imposes on it cytoplasmic location and prevents its interaction with the NF-κB-binding DNA sequence and activation of transcription. The dissociation of I-κB from the NF-κB dimer constitutes the critical step of its activation by many of its inducing agents (DiDonato et al., 1995). Knowledge of the

mechanisms that are involved in this regulation is still limited. There is also just little understanding of the way in which cell specificity in terms of responsiveness to the various NF-κB-inducing agents is determined.

One of the most potent inducing agents of NF-κB is the cytokine tumor necrosis factor (TNF). There are two different TNF receptors, the p55 and p75 receptors. Their expression levels vary independently among different cells (Vandenabeele et al., 1995). The p75 receptor responds preferentially to the cell-bound form of TNF (TNF is expressed both as a beta-transmembrane protein and as a soluble protein) while the p55 receptor responds just as effectively to soluble TNF molecules (Grell et al., 1995). The intracellular domains of the two receptors are structurally unrelated and bind different cytoplasmic proteins. Nevertheless, at least part of the effects of TNF, including the cytocidal effect of TNF and the induction of NF-κB, can be induced by both receptors. This feature is cell specific. The p55 receptor is capable of inducing a cytocidal effect or activation of NF-κB in all cells that exhibit such effects in response to TNF. The p75-R can have such effects only in some cells. Others, although expressing the p75-R at high levels, show induction of the effects only in response to stimulation of the p55-R (Vandenabeele et al., 1995). Apart from the TNF receptors, various other receptors of the TNF/NGF receptor family: CD30 (McDonald et al., 1995), CD40 (Berberich et al., 1994; Lalmanach-Girard et al., 1993), the lymphotoxin beta receptor and, in a few types of cells, Fas/APO1 (Rensing-Ehl et al., 1995), are also capable of inducing activation of NF-κB. The IL-1 type I receptor, also effectively triggering NF-κB activation, shares most of the effects of the TNF receptors despit the fact that it has no structural similarity to them.

The activation of NF-κB upon triggering of these various receptors results from induced phosphorylation of its associated I-κB molecules. This phosphorylation tags I-κB to degradation, which most likely occurs in the proteasome. The nature of the kinase that phosphorylates I-κB, and its mechanism of activation upon receptor triggering is still unknown. However, in the recent two years some knowledge has been gained as to the identity of three receptor-associated proteins that appear to take part in initiation of the phosphorylation (see diagrammatic illustration in Figures 2a and 6). A protein called TRAF2, initially cloned by D. Goeddel and his colleagues (Rothe et al., 1994), seems to play a central role in NF-κB-activation by the various receptors of the TNF/NGF family. The protein, which when expressed at high levels can by itself trigger NF-κB activation,

binds to activated p75 TNF-R (Rothe et al., 1994), lymphotoxin beta receptor (Mosialos et al., 1995), CD40 (Rothe et al., 1995a) and CD-30 (unpublished data) and mediates the induction of NF-κB by them. TRAF2 does not bind to the p55 TNF receptor nor to Fas/APO1, however, it can bind to a p55 receptor-associated protein called TRADD and TRADD has the ability to bind to a Fas/APO1-associated protein called MORT1 (or FADD - see Boldin et al. 1995b and 1996). Another receptor-interacting protein, called RIP (see Stanger et al., 1995) is also capable of interacting with TRAF2 as well as with FAS/APO1, TRADD, the p55 TNF receptor and MORT-1. Thus, while RIP has been associated with cell cytotoxicity induction (cell death), its ability to interact with TRAF2 also implicates it in NF-κB activation and it also may serve in addition to augment the interaction between FAS/APO1, MORT-1, p55 TNF receptor and TRADD with TRAF2 in the pathway leading to NF-κB activation. These associations apparently allow the p55 TNF receptor and Fas/APO1 to trigger NF-κB activation (Hsu et al., 1995; Boldin et al., 1995; Chinnalyan et al., 1995; Varfolomeev et al., 1996; Hsu et al., 1996). The triggering of NF-κB activation by the IL-1 receptor occurs independently of TRAF2 and may involve a recently-cloned IL-1 receptor-associated protein-kinase called IRAK (Croston et al., 1995).

By what mechanism TRAF2 acts is not clear. Several cytoplasmic molecules that bind to TRAF2 have been identified (Rothe et al., 1994; Rothe et al., 1995b). However, the information on these molecules does not provide any clue as to the way by which TRAF2, which by itself does not possess any enzymatic activity, triggers the phosphorylation of I-κB. There is also no information yet of mechanisms that dictate cell-specific pattern of activation of TRAF2 by different receptors, such as observed for the induction of NF-κB by the two TNF receptors.

In addition to the above mentioned, of the various TRAF proteins, it should also be noted that TRAF2 binds to the p55 (CD120a) and p75 (CD120b) TNF receptors, as well as to several other receptors of the TNF/NGF receptor family, either directly or indirectly via other adpator proteins as noted above, for example with reference to the FAS/APO1 receptor, and the adaptor proteins MORT-1, TRADD and RIP. As such, TRAF2 is crucial for the activation of NF-κB (see also Wallach, 1996). However, TRAF3 actually inhibits activation of NF-κB by some receptors of the TNF/NGF family (see Rothe et al., 1995a), whilst TRAF6 is required for induction of NF-κB by IL-1 (see Cao et al., 1996a).

Accordingly, as regards NF-κB activation and its importance in maintaining cell viability, the various intracellular pathways involved in this activation have heretofore not been clearly elucidated, for example, how the various TRAF proteins, are involved directly or indirectly.

Furthermore, as is now known regarding various members of the TNF/NGF receptor family and their associated intracellular signaling pathways inclusive of various adaptor, mediator/modulator proteins (see brief reviews and references in, for example, co-pending co-owned Israel Patent Application Nos. 114615, 114986, 115319, 116588), TNF and the FAS/APO1 ligand, for example, can have both beneficial and deleterious effects on cells. TNF, for example, contributes to the defence of the organism against tumors and infectious agents and contributes to recovery from injury by inducing the killing of tumor cells and virus-infected cells, augmenting antibacterial activities of granulocytes, and thus in these cases the TNF-induced cell killing is desirable. However, excess TNF can be deleterious and as such TNF is known to play a major pathogenic role in a number of diseases such as septic shock, anorexia, rheumatic diseases, inflammation and graft-vs-host reactions. In such cases TNF-induced cell killing is not desirable. The FAS/APO1 ligand, for example, also has desirable and deleterious effects. This FAS/APO1 ligand induces via its receptor the killing of autoreactive T cells during maturation of T cells, i.e. the killing of T cells which recognize self-antigens, during their development and thereby preventing autoimmune diseases. Further, various malignant cells and HIV-infected cells carry the FAS/APO1 receptor on their surface and can thus be destroyed by activation of this receptor by its ligand or by antibodies specific thereto, and thereby activation of cell death (apoptosis) intracellular pathways mediated by this receptor. However, the FAS/APO1 receptor may mediate deleterious effects, for example, uncontrolled killing of tissue which is observed in certain diseases such as acute hepatitis that is accompanied by the destruction of liver cells.

In view of the above, namely, that receptors of the TNF/NGF family can induce cell death pathways on the one hand and can induce cell survival pathways (via NF-κB induction) on the other hand, there apparently exists a fine balance, intracellularly between these two opposing pathways. For example, when it is desired to achieve maximal destruction of cancer cells or other infected or diseased cells, it would be desired to have TNF and/or the FAS/APO1 ligand inducing only the cell death pathway without inducing

NF-κB. Conversely, when it is desired to protect cells such as in, for example, inflammation, graft-vs-host reactions, acute hepatitis, it would be desirable to block the cell killing induction of TNF and/or FAS/APO1 ligand and enhance, instead, their induction of NF-κB. Likewise, in certain pathological circumstances it would be desirable to block the intracellular signaling pathways mediated by the p75 TNF receptor and the IL-1 receptor, while in others it would be desirable to enhance these intracellular pathways.

Summary of the Invention

It is an object of the invention to provide novel proteins, including all isoforms, analogs, fragments or derivatives thereof which are capable of binding to the tumor necrosis factor receptor-associated (TRAF) proteins. As the TRAF proteins are involved in the modulation or mediation of the activation of the transcription factor NF-κB, which is initiated by some of the TNF/NGF receptors, as well as others as noted above, the novel proteins of the present invention by binding to TRAF proteins are therefore capable of affecting (modulating or mediating) the intracellular signaling processes initiated by various ligands (e.g. TNF, FAS ligand and others) binding to their receptors such as, for example, their modulation/mediation of NF-κB activation, via interaction directly or indirectly with TRAF proteins.

The novel proteins of the present invention are therefore direct modulators/mediators of the intracellular biological activity of TRAF proteins (e.g. induction of NF-κB activation by TRAF2 and TRAF6 and inhibition of NF-κB activation, by TRAF3).

The novel proteins of the invention are likewise indirect modulators/mediators of the intracellular biological activity of a variety of other proteins which are capable of interacting with TRAF proteins directly or indirectly (e.g. FAS/APO1 receptor, p55 TNF receptor, p75 TNF receptor, IL-1 receptor and their associated proteins, such as, for example, MORT-1, TRADD, RIP).

Another object of the invention is to provide antagonists (e.g. antibodies, peptides, organic compounds, or even some isoforms) to the above novel TRAF-binding proteins, including isoforms, analogs, fragments and derivatives thereof, which may be used to inhibit the signaling process, or, more specifically, to inhibit the activation of NF-κB and its associated involvement in cell-survival processes, when desired. Likewise, when the TRAF-

binding proteins of the invention or the TRAF protein to which they bind (e.g. TRAF3) are themselves inhibitory for NF-κB activation, then it is an object to provide antagonists to these TRAF-binding proteins to activate the signaling process or more specifically, to block the inhibition of NF-κB activation and hence bring about enhanced NF-κB activation, when desired.

A further object of the invention is to use the above novel TRAF-binding proteins, isoforms, analogs, fragments and derivatives thereof, to isolate and characterize additional proteins or factors, which may be involved in regulation of TRAF protein activity and/or the above noted receptor activity, e.g. other proteins which may bind to TRAF proteins and influence their activity, and/or to isolate and identify other receptors or other cellular proteins further upstream or downstream in the signaling process(es) to which these novel proteins, analogs, fragments and derivatives bind, and hence, in whose function they are also involved.

A still further object of the invention is to provide inhibitors which can be introduced into cells to bind or interact with the novel TRAF-binding proteins and possible isoforms thereof, which inhibitors may act to inhibit TRAF protein-associated activity in, for example, NF-κB activation and hence, when desired, to inhibit NF-κB activation; or which may act to inhibit inhibitory TRAF-associated activity (e.g. TRAF3) in NF-κB activation and hence, when desired, to enhance NF-κB activation.

Moreover, it is an object of the present invention to use the above-mentioned novel TRAF-binding proteins, isoforms and analogs, fragments and derivatives thereof as antigens for the preparation of polyclonal and/or monoclonal antibodies thereto. The antibodies, in turn, may be used, for example, for the purification of the new proteins from different sources, such as cell extracts or transformed cell lines.

Furthermore, these antibodies may be used for diagnostic purposes, e.g. for identifying disorders related to abnormal functioning of cellular effects mediated directly by TRAF proteins or mediated by the p55 TNF receptor, FAS/APO1 receptor, or other related receptors and their associated cellular proteins (e.g. MORT-1, TRADD, RIP), which act directly or indirectly to modulate/mediate intracellular processes via interaction with TRAF proteins.

A further object of the invention is to provide pharmaceutical compositions comprising the above novel TRAF proteins, isoforms, or analogs, fragments or derivatives

thereof, as well as pharmaceutical compositions comprising the above noted antibodies or other antagonists.

In accordance with the present invention, a number of novel TRAF-binding proteins, in particular, TRAF2-binding proteins, have been isolated. These TRAF2-binding proteins have high specificity of binding to TRAF2 (see Examples below) and hence are modulators or mediators of TRAF2 intracellular activity. TRAF2 is involved in the modulation or mediation of at least one intracellular signaling pathway being the cell survival- or viability- related pathway in which Traf 2 is directly involved in activation of NF-κB which plays a central role in cell survival. In fact, one of these new proteins, called NIK (for 'NF-κB inducing kinase') binds to TRAF2 and stimulates NF-κB activity. NIK is a kinase sharing sequence similarity with several MAPKK kinases (see below). Further, TRAF2 by being capable of interaction directly or indirectly with the above noted p55 TNF receptor, p75 TNF receptor, FAS/APO1 receptors and their associated proteins MORT-1, TRADD and RIP, also is a mediator or modulator of the NF-κB induction or activation activity attributed to these receptors. TRAF2 is therefore a modulator/mediator of the cell survival pathways (as opposed to the cell death pathways) mediated by these receptors and their associated proteins and as such the extent of interaction between these receptors and/or proteins with TRAF2 is an important factor in the outcome of the activity of these receptors (once activated by their ligands), namely, whether the cells will survive or die. Accordingly, the proteins of the invention, for example, NIK, play a key role in this interaction between TRAF2 and the other proteins/receptors with which TRAF2 interacts, as proteins such as NIK by binding specifically to TRAF2 will modulate its activity and/or will have their activity modulated by interaction with TRAF2.

The TRAF-binding proteins, such as, for example, the TRAF2-binding proteins, including NIK, have been isolated and cloned using the two-hybrid system, partially and fully sequenced, and characterized, and as is detailed herein below appear to be highly specific TRAF2-binding proteins, and hence specific TRAF2 modulators/mediators.

As will be used herein throughout, TRAF protein activity, for example TRAF2 activity, is meant to include its activity in modulation/mediation in the cell survival pathway, especially as concerns NF-κB induction/activation. Likewise, as used herein throughout TRAF-binding protein, in particular TRAF2-binding protein, activity is meant to include their modulation/mediation of TRAF-, in particular, TRAF2- activity by virtue of

their specific binding to TRAF, especially TRAF2 proteins, this modulation/mediation including modulation/mediation of cell survival pathways, in particular, those relating to NF-κB activation/induction in which TRAF proteins, especially TRAF2 is involved directly or indirectly and as such TRAF or TRAF2-binding protein may be considered as indirect modulator/mediators of all the above mentioned proteins and possibly a number of others which are involved in cell survival, especially NF-κB activation/induction and to which TRAF2 (or other TRAF proteins) binds, or with which TRAF2 (or other TRAF proteins) interacts in a direct or indirect fashion.

Accordingly, the present invention provides a DNA sequence encoding a protein capable of binding to a tumor necrosis factor receptor-associated (TRAF) molecule.

One embodiment of the DNA sequence of the invention is a sequence encoding a protein capable of binding to TRAF2.

Another embodiment of the DNA sequence of the invention is a sequence encoding a protein capable of binding to at least the amino acid residues 222-501 of the amino acid sequence of TRAF2

Other embodiments of the DNA sequence of the invention include :

(a) a cDNA sequence of the herein designated clone 9 comprising the nucleotide sequence depicted in Fig 3a.;

(b) a cDNA sequence of the herein designated clone 10 comprising the nucleotide sequence depicted in Fig 4;

(c) a cDNA sequence of the herein designated clone 15 comprising the nucleotide sequence depicted in Fig. 5a;

(d) a fragment of a sequence (a)-(c) which encodes a biologically active protein capable of binding to least the 222-501 amino acid sequence of TRAF2;

(e) a DNA sequence capable of hybridization to a sequence of (a)-(d) under moderately stringent conditions and which encodes a biologically active protein capable of binding to at least the 222-501 amino acid sequence of TRAF2; and

(f) a DNA sequence which is degenerate as a result of the genetic code to the DNA sequences defined in (a)-(e) and which encodes a biologically active protein capable of binding to at least the 222-501 amino acid sequence of TRAF2.

Yet other embodiments of the DNA sequence of the invention noted above include :

A DNA sequence selected from the sequences contained in the herein designated cDNA clones 9 and 15;

A DNA sequence which encodes a protein that also modulates NF-κB activity; and

A DNA sequence selected from the sequences contained in the herein designated cDNA clone 10.

An additional preferred embodiment of the above DNA sequences of the invention is a DNA sequence comprising the DNA sequence encoding the protein NIK (for 'NF-κB inducing kinase').

Embodiments of the above DNA sequence of the invention encoding the protein NIK include :

(i) A DNA sequence encoding the protein NIK, isoforms, fragments or analogs thereof, said NIK, isoforms, fragments or analogs thereof being capable of binding to TRAF2 and which is capable of modulating the activity of NF-κB;

(ii) A DNA sequence as in (i) above, selected from the group consisting of :

a) a cDNA sequence derived from the coding region of a native NIK protein;

b) DNA sequences capable of hybridization to a sequence of (a) under moderately stringent conditions and which encode a biologically active NIK; and

c) DNA sequences which are degenerate as a result of the genetic code to the sequences defined in (a) and (b) and which encode a biologically active NIK protein;

(iii) A DNA sequence as in (i) or (ii) above comprising at least part of the sequence depicted in Fig. 6 and encoding at least one active NIK protein, isoform, analog or fragment;

(iv) A DNA sequence as in (iii) above encoding a NIK protein, isoform, analog or fragment having at least part of the amino acid sequence depicted in Fig. 6.

In another aspect, the invention provides proteins or polypeptides encoded by the above noted DNA coding sequences of the invention, the isoforms, analogs, fragments and derivatives of said proteins and polypeptides, provided that they are capable of binding to TRAF2, preferably to at least the 222-501 amino acid sequenced of TRAF2. Embodiments of these proteins/polypeptides, and their isoforms, analogs, fragments and derivatives according to the invention include :

(a) a protein being the protein encoded by herein designated clone 10;

(b) a protein, isoforms, fragments, analogs and derivatives thereof, being the NIK protein, isoforms, analogs, fragments and derivatives thereof encoded by the above noted DNA sequences encoding said NIK protein, isoforms, analogs, fragments and derivatives; and

(c) a NIK protein, isoforms, analogs, fragments and derivatives thereof being the NIK protein, isoforms, analogs, fragments and derivatives thereof encoded by the above noted DNA sequences encoding said NIK protein, isoforms, analogs, fragments and derivatives, wherein said protein, isoforms, fragments and derivatives have at least part of the amino acid sequence depicted in Fig. 6.

In yet another aspect, the invention provides a vector comprising any of the above DNA sequences according to the invention which are capable of being expressed in host cells selected from prokaryotic and eukaryotic cells; and the transformed prokaryotic and eukaryotic cells containing said vector.

The invention also provides a method for producing a protein, isoform, analog, fragment or derivative encoded by any of the above DNA sequences according to the invention which comprises growing the above mentioned transformed host cells under conditions suitable for the expression of said protein, isoforms, analogs, fragments or derivatives, effecting post-translational modification, as necessary, for obtaining said protein, isoform, analogs, fragments or derivatives and isolating said expressed protein, isoforms, analogs, fragments or derivatives.

In a further aspect, the invention provides antibodies or active fragments or derivatives thereof, specific for the above TRAF-binding proteins, analogs, isoforms, fragments or derivatives thereof or specific for the NIK protein, isoform, analog, fragment or derivative thereof noted above.

In a different aspect, the invention provides the following screening methods:

(i) A method for screening of a ligand capable of binding to a protein according to the invention, as noted above, including isoforms, analogs, fragments or derivatives thereof, comprising contacting an affinity chromatography matrix to which said protein, isoform, analog, fragment or derivative is attached with a cell extract whereby the ligand is bound to said matrix, and eluting, isolating and analyzing said ligand.

(ii) A method for screening of a DNA sequence coding for a ligand capable of binding to a protein, isoform, analog, fragment or derivative according to the invention as noted above, comprising applying the yeast two-hybrid procedure in which a sequence encoding said protein, isoform analog, derivative or fragment is carried by one hybrid vector and sequences from a cDNA or genomic DNA library are carried by the second hybrid vector, transforming yeast host cells with said vectors, isolating the positively transformed cells, and extracting said second hybrid vector to obtain a sequence encoding said ligand.

Similarly, there is also provided a method for isolating and identifying proteins, isoforms, analogs, fragments according to the invention noted above, capable of binding directly to TRAF2, comprising applying the yeast two-hybrid procedure in which a sequence encoding said TRAF2 is carried by one hybrid vector and sequence from a cDNA or genomic DNA library is carried by the second hybrid vector, the vectors then being used to transform yeast host cells and the positive transformed cells being isolated, followed by extraction of the said second hybrid vector to obtain a sequence encoding a protein which binds to said TRAF2.

In yet another aspect of the invention there is provided a method for the modulation or mediation in cells of the activity of NF-κB or any other intracellular signaling activity modulated or mediated by TRAF2 or by other molecules to which a protein, isoform, analog, fragment or derivative thereof of the invention as noted above, said method comprising treating said cells by introducing into said cells one or more of said protein, isoform, analog, fragment or derivative thereof in a form suitable for intracellular introduction thereof, or introducing into said cells a DNA sequence encoding said one or more protein, isoform, analog, fragment or derivative thereof in the form of a suitable vector carrying said sequence, said vector being capable of effecting the insertion of said sequence into said cells in a way that said sequence is expressed in said cells.

Embodiments of this above method for modulation/mediation in cells of the activity of NF-κB or any other intracellular signaling activity modulated or mediated by TRAF2 or other molecules include :

(i) A method as above, wherein said treating of cells comprises introducing into said cells a DNA sequence encoding said protein, isoform, fragment, analog or derivative in the form of a suitable vector carrying said sequence, said vector being capable of effecting the

insertion of said sequence into said cells in a way that said sequence is expressed in said cells.

(ii) A method as above wherein said treating of said cells is by transfection of said cells with a recombinant animal virus vector comprising the steps of :

(a) constructing a recombinant animal virus vector carrying a sequence encoding a viral surface protein (ligand) that is capable of binding to a specific cell surface receptor on the surface of said cells to be treated and a second sequence encoding a protein selected from the said protein, isoforms, analogs, fragments and derivatives according to the invention,that when expressed in said cells is capable of modulating/mediating the activity of NF-κB or any other intracellular signaling activity modulated/mediated by TRAF2 or other said molecules; and

(b) infecting said cells with said vector of (a).

Likewise, the present invention also provides a method for modulating TRAF2 modulated/mediated effect on cells comprising treating said cells with the antibodies or active fragments or derivatives thereof, according to the invention as noted above, said treating being by application of a suitable composition containing said antibodies, active fragments or derivatives thereof to said cells, wherein when the TRAF2-binding protein or portions thereof of said cells are exposed on the extracellular surface, said composition is formulated for extracellular application, and when said TRAF2-binding proteins are intracellular said composition is formulated for intracellular application.

Other methods of the invention for modulating the TRAF2 modulated/mediated effect on cells include :

(i) A method comprising treating said cells with an oligonucleotide sequence encoding an antisense sequence for at least part of the DNA sequence encoding a TRAF2-binding protein, this DNA sequence being any of the above mentioned ones of the invention, said oligonucleotide sequence being capable of blocking the expression of the TRAF2-binding protein.

(ii) A method as in (i) above wherein said oligonucleotide sequence is introduced to said cells via a recombinant virus as noted above, wherein said second sequence of said virus encodes said oligonucleotide sequence.

(iii) A method comprising applying the ribozyme procedure in which a vector encoding a ribozyme sequence capable of interacting with a cellular mRNA sequence

encoding a TRAF2-binding protein, isoform, analog, fragment or derivative of the invention noted above, is introduced into said cells in a form that permits expression of said ribozyme sequence in said cells, and wherein when said ribozyme sequence is expressed in said cells it interacts with said cellular mRNA sequence and cleaves said mRNA sequence resulting in the inhibition of expression of said TRAF2-binding protein in said cells.

It should be noted that for all the above methods of the invention the protein of the invention as indicated, can be specifically NIK or at least one of the NIK isoforms, analogs, fragments and derivatives thereof.

In the above methods and embodiments thereof of the invention there is included also a method for the prevention or treatment of a pathological condition associated with NF-κB induction or with any other activity mediated by TRAF2 or by other molecules to which a protein, isoform, analog, fragment or derivative, according to the invention, binds, said method comprising administering to a patient in need an effective amount of a protein, isoform, analog, fragment or derivative, according to the invention, or a DNA molecule coding therefor, or a molecule capable of disrupting the interaction of said protein, isoform, analog, fragment or derivative, with TRAF2 or any other molecule to which said protein, isoform, analog, fragment or derivative binds. In this method of the invention, said protein of the invention administered to the patient in need can be specifically the protein encoded by clone 10, NIK, an isoform, analog, derivative or fragment of NIK, or a DNA molecule coding therefor. The protein encoded by clone 10 acts to inhibit NF-κB induction, as do other fragments of NIK, while NIK induces NF-κB activation.

In an additional aspect of the invention there is provided a pharmaceutical composition for the modulation of the TRAF2 modulated/mediated effect on cells comprising, as active ingredient at least one of the TRAF2-binding proteins, according to the invention, its biologically active fragments, analogs, derivatives or mixtures thereof.

Other pharmaceutical compositions or embodiments thereof according to the invention include :

(i) A pharmaceutical composition for modulating the TRAF2 modulated/mediated effect on cells comprising, as active ingredient, a recombinant animal virus vector encoding a protein capable of binding a cell surface receptor and encoding at least one TRAF2-binding protein, isoform, active fragments or analogs, according to the invention.

(ii) A pharmaceutical composition for modulating the TRAF2 modulated/mediated effect on cells comprising as active ingredient, an oligonucleotide sequence encoding an anti-sense sequence of the TRAF2-binding protein mRNA sequence according to the invention.

A further embodiment of the above pharmaceutical composition is specifically a pharmaceutical composition for the prevention or treatment of a pathological condition associated with NF-κB induction or with any other activity mediated by TRAF2 or by other molecules to which a protein, analog, isoform, fragment or derivative, according to the invention binds, said composition comprising an effective amount of a protein, analog, isoform, fragment or derivative, according to the invention or a DNA molecule coding therefor, or a molecule capable of disrupting the interaction of said protein, analog, isoform, fragment or derivative, with TRAF2 or any other molecule to which said protein, analog, isoform, fragment or derivative, binds. In a yet further specific embodiment said pharmaceutical composition comprising an effective amount of the protein encoded by clone 10, NIK, an isoform, analog, derivative or fragment of NIK, or a DNA molecule coding therefor.

In yet another specific embodiment, the invention provides a pharmaceutical composition for the prevention or treatment of a pathological condition associated with NF-κB induction or with any other activity mediated by TRAF2 or by other molecules to which the protein NIK binds, said composition comprising a molecule capable of interfering with the protein kinase activity of NIK. In this composition, the interfering molecule may be an effective amount of NIK mutated in active site residues, this mutated NIK serving to interfere with native NIK, in particular, the kinase activity of NIK.

One known condition associated with NF-κB induction (abnormal) is AIDS, others are e.g. autoimmune diseases, as well as tumors.

Still further aspects and embodiments of the invention are :

(i) A method for identifying and producing a ligand capable of modulating the cellular activity modulated/mediated by TRAF2 comprising :

a) screening for a ligand capable of binding to a polypeptide comprising at least a portion of TRAF2 having the amino acid residues 221-501 of TRAF2;

b) identifying and characterizing a ligand, other than TRAF2 or portions of a receptor of the TNF/NGF receptor family, found by said screening step to be capable of said binding; and

c) producing said ligand in substantially isolated and purified form.

(ii) A method for identifying and producing a ligand capable of modulating the cellular activity modulated/mediated by a protein, isoform, analog, fragment or derivative, according to the invention, comprising :

a) screening for a ligand capable of binding to a polypeptide comprising at least a portion of the NIK sequence depicted in Fig. 6;

b) identifying and characterizing a ligand, other than TRAF2 or portions of a receptor of the TNF/NGF receptor family, found by said screening step to be capable of said binding; and

c) producing said ligand in substantially isolated and purified form.

(iii) A method for identifying and producing a ligand capable of modulating the cellular activity modulated/mediated by NIK comprising :

a) screening for a ligand capable of binding to a polypeptide comprising at least a portion of the NIK sequence depicted in Fig. 6;

b) identifying and characterizing a ligand, other than TRAF2 or portions of a receptor of the TNF/NGF receptor family, found by said screening step to be capable of said binding; and

c) producing said ligand in substantially isolated and purified form.

(iv) A method for identifying and producing a ligand capable of directly or indirectly modulating the cellular activity modulated/mediated by NIK comprising :

a) screening for a molecule capable of modulating activities modulated/mediated by NIK;

b) identifying and characterizing said molecule; and

c) producing said molecule in substantially isolated and purified form.

(v) A method for identifying and producing a molecule capable of directly or indirectly modulating the cellular activity modulated/mediated by a protein, isoform, analog, fragment or derivative of the invention, comprising :

a) screening for a molecule capable of modulating activities modulated/mediated by a protein, isoform, analog, fragment or derivative according to the invention;

b) identifying and characterizing said molecule; and

c) producing said molecule in substantially isolated and purified form.

Other aspects and embodiments of the present invention are also provided as arising from the following detailed description of the invention.

It shoud be noted that, where used throughout, the following terms : "modulation/mediation of the TRAF (or TRAF2) effect on cells" and any other such "modulation/mediation" mentioned in the specification are understood to encompass *in vitro* as well as *in vivo* treatment and, in addition, also to encompass inhibition or enhancement/augmentation.

Brief Description of the Drawings

Fig. 1 shows a diagramatic illustration of the structure of the TRAF2 molecule.

Fig. 2a-b shows schematic diagrams illustrating some of the proteins involved in NF-kB activation, including the new TRAF-binding proteins of the present invention (e.g. NIK), in which (a) is a partial scheme and (b) is a more complete scheme;

Figs. 3a-b show the nucleotide sequence of the 5' end of clone 9 (a) and the deduced amino acid sequenced encoded thereby (b);

Fig. 4 shows the nucleotide sequence of clone 10;

Figs. 5a-b show the nucleotide sequence of clone 15 (a) and the deduced amino acid sequence encoded thereby (b);

Fig. 6 shows the nucleotide sequence and the deduced amino acid sequence of NIK; and

Fig. 7 shows an alignment of the sequence of protein NIK with the sequence of the mouse protein kinase mMEKK (mouse MAPK or ERK Kinase Kinase) and a number of other kinases. The regions corresponding to the conserved motifs I to XI in protein kinases are marked.

Detailed Description of the Invention

The present invention relates to DNA sequences encoding proteins capable of binding to a tumor necrosis factor receptor-associated factor (TRAF) molecule, and the proteins encoded thereby.

In a prefered embodiment, the present invention concerns cDNA sequences herein designated clone 9, clone 10 and clone 15 (depicited in Figs. 3a, 4 and 5a, respectively), which encode for proteins capable of binding to TRAF2, and the proteins encoded by those DNA sequences.

In a further preferred embodiment the invention relates to the DNA sequence encoding the NIK protein, and the NIK protein itself.

The DNA and the deduced amino acid sequences mentioned above represent new sequences; they do not appear in the 'GENEBANK' or 'PROTEIN BANK' data banks of DNA or amino acid sequences.

Within the scope of the present invention are also fragments of the above mentioned DNA sequences and DNA sequences capable of hybridization to those sequences or part of them, under moderately stringent conditions, provided they encode a biologically active protein or polypeptide capable of binding to at least the 222-501 amino acid sequence of TRAF2.

The present invention also concerns a DNA sequence which is degenerate as a result of the genetic code to the above mentioned DNA sequences and which encodes a biologically active protein or polypeptide capable of binding to at least the 222-501 amino acid sequence of TRAF2.

As regards TRAF2, it should be noted that several members of the TNF/NGF receptor family activate the transcription factor NF-κB by direct or indirect association with TRAF2, which is thus an adaptor protein for these receptors and may thus also be considered as a modulator/mediator of the induction of NF-κB activation activity of these TNF/NGF receptors (see the scheme in Fig. 2b). Another receptor, the IL-1 receptor activates NF-κB independently of TRAF2. One of the embodiments of a preferred TRAF2-binding protein in accordance with the present invention is the NIK protein, which binds NIK in a very specific way and stimulates NF-κB activity. NIK is a serine/threonine kinase having sequence similarity with several MAPKK kinases (see Examples below). NIK analogs or muteins produced in accordance with the present invention (see Examples)

which lack the kinase activity of NIK fail to stimulate NF-κB activation, when these analogs/muteins are expressed in cells. Further, such NIK analogs/muteins when expressed in cells also block NF-κB induction by TNF as well as by other inducing agents such as the bacterial endotoxin LPS, forbol myristate acetate (a protein kinase C activator), and the HTLV-1 protein TAX. TNF induction of NF-κB activity is via either of the two TNF receptors (p55 and p75 TNF receptors) and hence it appears that the NIK mutein/analogs block induction of NF-κB activation via these receptors. Likewise, TNF and the FAS/APO1 receptor ligand may also induce NF-κB activity via a related receptor, the FAS/APO1 receptor, which induction is also blocked by NIK muteins/analogs. Moreover, the above receptors have adaprot proteins TRADD, RIP and MORT1 which can all also induce NF-κB activity, but which induction is also blocked by NIK muteins/analogs. In addition, such NIK muteins/analogs also blocked NF-κB induction by IL-1 (functioning via the IL-1 receptor). Accordingly, it arises that NIK participates in an NF-κB-inducing cascade that is common to receptors of the TNF/NGF family and to the IL-1 receptor. NIK also appears to act in a direct way in inducing NF-κB activation possibly by enhancing I-κB phosphorylation directly. This arises from the present observations that the above NIK analogs/muteins lacking kinase activity (also called dominant-negative mutants) when expressed in cells did not effect in any manner the TNF-induced activation of Jun kinase, indicating that NIK acts specifically to enhance phosphorylation of I-κB without affecting the MAP kinase involved in Jun phosphorylation.

Thus, the present invention concerns the DNA sequences encoding biologically active TRAF-binding proteins, e.g. TRAF2-binding proteins, such as, for example, NIK, as well as analogs, fragments and derivatives thereof, and the analogs, fragments and derivatives of the proteins encoded thereby. The preparation of such analogs, fragments and derivatives is by standard procedures (see for example, Sambrook et al., 1989) in which in the DNA encoding sequences, one or more codons may be deleted, added or substituted by another, to yield encoded analogs having at least a one amino acid residue change with respect to the native protein. Acceptable analogs are those which retain at least the capability of binding to TRAF2 with or without mediating any other binding or enzymatic activity, e.g. analogs which bind TRAF2 but do not signal, i.e. do not bind to a further downstream protein or other factor, or do not catalyze a signal-dependent reaction. In such a way analogs can be produced which have a so-called dominant-negative effect,

namely, an analog which is defective either in binding to TRAF2, or in subsequent signaling following such binding as noted above. Such analogs can be used, for example, to inhibit the CD40, p55 TNF and p75 TNF (FAS/APO1 and other related receptor effects, as well as effected mediated by various receptor associated proteins (adaptors) as noted above, by competing with the natural TRAF2-binding proteins. Likewise, so-called dominant-positive analogs may be produced which would serve to enhance the TRAF2 effect. These would have the same or better TRAF2-binding properties and the same or better signaling properties of the natural TRAF2-binding proteins. In an analogous fashion, biologically active fragments of the clones of the invention may be prepared as noted above with respect to the preparation of the analogs. Suitable fragments of the DNA sequences of the invention are those which encode a protein or polypeptide retaining the TRAF2 binding capability or which can mediate any other binding or enzymatic activity as noted above. Accordingly, fragments of the encoded proteins of the invention can be prepared which have a dominant-negative or a dominant-positive effect as noted above with respect to the analogs. Similarly, derivatives may be prepared by standard modifications of the side groups of one or more amino acid residues of the proteins, their analogs or fragments, or by conjugation of the proteins, their analogs or fragments, to another molecule e.g. an antibody, enzyme, receptor, etc., as are well known in the art.

Of the above DNA sequences of the invention which encode a TRAF-binding protein, (e.g. TRAF2-binding protein, such as for example, NIK) isoform, analog, fragment or derivative, there is also included, as an embodiment of the invention, DNA sequences capable of hybridizing with a cDNA sequence derived from the coding region of a native TRAF-binding protein, in which such hybridization is performed under moderately stringent conditions, and which hybridizable DNA sequences encode a biologically active TRAF-binding protein. These hybridizable DNA sequences therefore include DNA sequences which have a relatively high homology to the native TRAF-binding proteins cDNA sequence, (e.g. TRAF2-binding protein cDNA sequence, such as, for example the NIK cDNA sequence) and as such represent TRAF-binding protein-like sequences which may be, for example, naturally-derived sequences encoding the various TRAF-binding protein isoforms, or naturally-occuring sequences encoding proteins belonging to a group of TRAF-binding protein-like sequences encoding a protein having the activity of TRAF-binding proteins (e.g. TRAF2-binding proteins, such as, for example, NIK). Further, these

sequences may also, for example, include non-naturally occuring, synthetically produced sequences, that are similar to the native TRAF-binding protein cDNA sequence but incorporate a number of desired modifications. Such synthetic sequences therefore include all of the possible sequences encoding analogs, fragments and derivatives of TRAF-binding proteins (e.g. TRAF2-binding proteins, such as, for example NIK), all of which have the activity of TRAF-binding proteins.

To obtain the various above noted naturally occuring TRAF-binding protein-like sequences, standard procedures of screening and isolation of naturally-derived DNA or RNA samples from various tissues may be employed using the natural TRAF-binding protein cDNA or portion thereof as probe (see for example standard procedures set forth in Sambrook et al., 1989).

Likewise, to prepare the above noted various synthetic TRAF-binding protein-like sequences encoding analogs, fragments or derivatives of TRAF-binding proteins (e.g. TRAF2-binding proteins, such as, for example NIK), a number of standard procedures may be used as are detailed herein below concerning the preparation of such analogs, fragments and derivatives.

A polypeptide or protein "substantially corresponding" to TRAF-binding protein includes not only TRAF-binding protein but also polypeptides or proteins that are analogs of TRAF-binding protein.

Analogs that substantially correspond to TRAF-binding protein are those polypeptides in which one or more amino acid of the TRAF-binding protein's amino acid sequence has been replaced with another amino acid, deleted and/or inserted, provided that the resulting protein exhibits substantially the same or higher biological activity as the TRAF-binding protein to which it corresponds.

In order to substantially correspond to TRAF-binding protein, the changes in the sequence of TRAF-binding proteins, such as isoforms are generally relatively minor. Although the number of changes may be more than ten, preferably there are no more than ten changes, more preferably no more than five, and most preferably no more than three such changes. While any technique can be used to find potentially biologically active proteins which substantially correspond to TRAF-binding proteins, one such technique is the use of conventional mutagenesis techniques on the DNA encoding the protein, resulting in a few modifications. The proteins expressed by such clones can then be screened for

their ability to bind to TRAF proteins (e.g. TRAF2) and to modulate TRAF protein (e.g. TRAF2) activity in modulation/mediation of the intracellular pathways noted above.

"Conservative" changes are those changes which would not be expected to change the activity of the protein and are usually the first to be screened as these would not be expected to substantially change the size, charge or configuration of the protein and thus would not be expected to change the biological properties thereof.

Conservative substitutions of TRAF-binding proteins include an analog wherein at least one amino acid residue in the polypeptide has been conservatively replaced by a different amino acid. Such substitutions preferably are made in accordance with the following list as presented in Table IA, which substitutions may be determined by routine experimentation to provide modified structural and functional properties of a synthesized polypeptide molecule while maintaining the biological activity characteristic of TRAF-binding protein.

Table IA

Original Residue	Exemplary Substitution
Ala	Gly;Ser
Arg	Lys
Asn	Gln;His
Asp	Glu
Cys	Ser
Gln	Asn
Glu	Asp
Gly	Ala;Pro
His	Asn;Gln
Ile	Leu;Val
Leu	Ile;Val
Lys	Arg;Gln;Glu
Met	Leu;Tyr;Ile
Phe	Met;Leu;Tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp;Phe
Val	Ile;Leu

Alternatively, another group of substitutions of TRAF-binding protein are those in which at least one amino acid residue in the polypeptide has been removed and a different residue inserted in its place according to the following Table IB. The types of substitutions which may be made in the polypeptide may be based on analysis of the frequencies of amino acid changes between a homologous protein of different species, such as those presented in Table 1-2 of Schulz et al., G.E., Principles of Protein Structure Springer-

Verlag, New York, NY, 1798, and Figs. 3-9 of Creighton, *T.E.*, Proteins: Structure and Molecular Properties, W.H. Freeman & Co., San Francisco, CA 1983. Based on such an analysis, alternative conservative substitutions are defined herein as exchanges within one of the following five groups:

TABLE IB

1. Small aliphatic, nonpolar or slightly polar residues: Ala, Ser, Thr (Pro, Gly);
2. Polar negatively charged residues and their amides: Asp, Asn, Glu, Gln;
3. Polar, positively charged residues: His, Arg, Lys;
4. Large aliphatic nonpolar residues: Met, Leu, Ile, Val (Cys); and
5. Large aromatic residues: Phe, Tyr, Trp.

The three amino acid residues in parentheses above have special roles in protein architecture. Gly is the only residue lacking any side chain and thus imparts flexibility to the chain. This however tends to promote the formation of secondary structure other than α-helical. Pro, because of its unusual geometry, tightly constrains the chain and generally tends to promote ß-turn-like structures, although in some cases Cys can be capable of participating in disulfide bond formation which is important in protein folding. Note that Schulz *et al., supra,* would merge Groups 1 and 2, above. Note also that Tyr, because of its hydrogen bonding potential, has significant kinship with Ser, and Thr, etc.

Conservative amino acid substitutions according to the present invention, e.g., as presented above, are known in the art and would be expected to maintain biological and structural properties of the polypeptide after amino acid substitution. Most deletions and substitutions according to the present invention are those which do not produce radical changes in the characteristics of the protein or polypeptide molecule. "Characteristics" is defined in a non-inclusive manner to define both changes in secondary structure, e.g. α-

helix or ß-sheet, as well as changes in biological activity, e.g., binding to TRAF proteins and/or mediation of TRAF proteins' effect on cell death.

Examples of production of amino acid substitutions in proteins which can be used for obtaining analogs of TRAF-binding proteins for use in the present invention include any known method steps, such as presented in U.S. patent RE 33,653, 4,959,314, 4,588,585 and 4,737,462, to Mark et al.; 5,116,943 to Koths et al., 4,965,195 to Namen et al.; 4,879,111 to Chong et al.; and 5,017,691 to Lee et al.; and lysine substituted proteins presented in U.S. patent No. 4,904,584 (Shaw et al.).

Besides conservative substitutions discussed above which would not significantly change the activity of TRAF-binding protein, either conservative substitutions or less conservative and more random changes, which lead to an increase in biological activity of the analogs of TRAF-binding proteins, are intended to be within the scope of the invention.

When the exact effect of the substitution or deletion is to be confirmed, one skilled in the art will appreciate that the effect of the substitution(s), deletion(s), etc., will be evaluated by routine binding and cell death assays. Screening using such a standard test does not involve undue experimentation.

At the genetic level, these analogs are generally prepared by site-directed mutagenesis of nucleotides in the DNA encoding the TRAF-binding protein, thereby producing DNA encoding the analog, and thereafter synthesizing the DNA and expressing the polypeptide in recombinant cell culture. The analogs typically exhibit the same or increased qualitative biological activity as the naturally occurring protein, Ausubel *et al.*, Current Protocols in Molecular Biology, Greene Publications *and Wiley Interscience, New York, NY*, 1987-1995; Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989.

Preparation of a TRAF-binding protein in accordance herewith, or an alternative nucleotide sequence encoding the same polypeptide but differing from the natural sequence due to changes permitted by the known degeneracy of the genetic code, can be achieved by site-specific mutagenesis of DNA that encodes an earlier prepared analog or a native version of a TRAF-binding protein. Site-specific mutagenesis allows the production of analogs through the use of specific oligonucleotide sequences that encode the DNA sequence of the desired mutation, as well as a sufficient number of adjacent nucleotides, to provide a primer sequence of sufficient size and sequence complexity to form a stable

duplex on both sides of the deletion junction being traversed. Typically, a primer of about 20 to 25 nucleotides in length is preferred, with about 5 to 10 complementing nucleotides on each side of the sequence being altered. In general, the technique of site-specific mutagenesis is well known in the art, as exemplified by publications such as Adelman *et al.*, *DNA* 2:183 (1983), the disclosure of which is incorporated herein by reference.

As will be appreciated, the site-specific mutagenesis technique typically employs a phage vector that exists in both a single-stranded and double-stranded form. Typical vectors useful in site-directed mutagenesis include vectors such as the M13 phage, for example, as disclosed by Messing *et al.*, *Third Cleveland Symposium on Macromolecules and Recombinant DNA*, Editor A. Walton, Elsevier, Amsterdam (1981), the disclosure of which is incorporated herein by reference. These phages are readily available commercially and their use is generally well known to those skilled in the art. Alternatively, plasmid vectors that contain a single-stranded phage origin of replication (Veira *et al.*, *Meth. Enzymol.* 153:3, 1987) may be employed to obtain single-stranded DNA.

In general, site-directed mutagenesis in accordance herewith is performed by first obtaining a single-stranded vector that includes within its sequence a DNA sequence that encodes the relevant polypeptide. An oligonucleotide primer bearing the desired mutated sequence is prepared synthetically by automated DNA/oligonucleotide synthesis. This primer is then annealed with the single-stranded protein-sequence-containing vector, and subjected to DNA-polymerizing enzymes such as *E. coli* polymerase I Klenow fragment, to complete the synthesis of the mutation-bearing strand. Thus, a mutated sequence and the second strand bears the desired mutation. This heteroduplex vector is then used to transform appropriate cells, such as *E. coli* JM101 cells, and clones are selected that include recombinant vectors bearing the mutated sequence arrangement.

After such a clone is selected, the mutated TRAF-binding protein sequence may be removed and placed in an appropriate vector, generally a transfer or expression vector of the type that may be employed for transfection of an appropriate host.

Accordingly, gene or nucleic acid encoding for a TRAF-binding protein can also be detected, obtained and/or modified, *in vitro*, *in situ* and/or *in vivo*, by the use of known DNA or RNA amplification techniques, such as PCR and chemical oligonucleotide synthesis. PCR allows for the amplification (increase in number) of specific DNA sequences by repeated DNA polymerase reactions. This reaction can be used as a

replacement for cloning; all that is required is a knowledge of the nucleic acid sequence... In order to carry out PCR, primers are designed which are complementary to the sequence of interest. The primers are then generated by automated DNA synthesis. Because primers can be designed to hybridize to any part of the gene, conditions can be created such that mismatches in complementary base pairing can be tolerated. Amplification of these mismatched regions can lead to the synthesis of a mutagenized product resulting in the generation of a peptide with new properties (i.e., site directed mutagenesis). See also, e.g., Ausubel, *supra*, Ch. 16. Also, by coupling complementary DNA (cDNA) synthesis, using reverse transcriptase, with PCR, RNA can be used as the starting material for the synthesis of the extracellular domain of a prolactin receptor without cloning.

Furthermore, PCR primers can be designed to incorporate new restriction sites or other features such as termination codons at the ends of the gene segment to be amplified. This placement of restriction sites at the 5' and 3' ends of the amplified gene sequence allows for gene segments encoding TRAF-binding protein or a fragment thereof to be custom designed for ligation other sequences and/or cloning sites in vectors.

PCR and other methods of amplification of RNA and/or DNA are well known in the art and can be used according to the present invention without undue experimentation, based on the teaching and guidance presented herein. Known methods of DNA or RNA amplification include, but are not limited to polymerase chain reaction (PCR) and related amplification processes (see, e.g., U.S. patent Nos. 4,683,195, 4,683,202, 4,800,159, 4,965,188, to Mullis *et al.*; 4,795,699 and 4,921,794 to Tabor *et al.*; 5,142,033 to Innis; 5,122,464 to Wilson *et al.*; 5,091,310 to Innis; 5,066,584 to Gyllensten *et al.*; 4,889,818 to Gelfand *et al.*; 4,994,370 to Silver *et al.*; 4,766,067 to Biswas; 4,656,134 to Ringold; and Innis *et al.*, eds., *PCR Protocols: A Guide to Method and Applications*) and RNA mediated amplification which uses anti-sense RNA to the target sequence as a template for double stranded DNA synthesis (U.S. patent No. 5,130,238 to Malek *et al.*, with the tradename NASBA); and immuno-PCR which combines the use of DNA amplification with antibody labeling (Ruzicka *et al.*, *Science* 260:487 (1993); Sano *et al.*, *Science* 258:120 (1992); Sano *et al.*, *Biotechniques* 9:1378 (1991)), the entire contents of which patents and reference are entirely incorporated herein by reference.

In an analogous fashion, biologically active fragments of TRAF-binding proteins (e.g. those of any of the TRAF2-binding proteins, such as, for example NIK) or its

isoforms) may be prepared as noted above with respect to the analogs of TRAF-binding proteins. Suitable fragments of TRAF-binding proteins are those which retain the TRAF-binding protein capability and which can mediate the biological activity of TRAF proteins or other proteins associated with TRAF proteins directly or indirectly. Accordingly, TRAF-binding protein fragments can be prepared which have a dominant-negative or a dominant-positive effect as noted above with respect to the analogs. It should be noted that these fragments represent a special class of the analogs of the invention, namely, they are defined portions of TRAF-binding proteins derived from the full TRAF-binding protein sequence (e.g., from that of any one of the TRAF2-binding proteins, such as, for example NIK or its isoforms), each such portion or fragment having any of the above-noted desired activities. Such fragment may be, e.g., a peptide.

Similarly, derivatives may be prepared by standard modifications of the side groups of one or more amino acid residues of the TRAF-binding protein, its analogs or fragments, or by conjugation of the TRAF-binding protein, its analogs or fragments, to another molecule e.g. an antibody, enzyme, receptor, etc., as are well known in the art. Accordingly, "derivatives" as used herein covers derivatives which may be prepared from the functional groups which occur as side chains on the residues or the N- or C-terminal groups, by means known in the art, and are included in the invention. Derivatives may have chemical moieties such as carbohydrate or phosphate residues, provided such a fraction has the same or higher biological activity as TRAF-binding proteins.

For example, derivatives may include aliphatic esters of the carboxyl groups, amides of the carboxyl groups by reaction with ammonia or with primary or secondary amines, N-acyl derivatives or free amino groups of the amino acid residues formed with acyl moieties (e.g., alkanoyl or carbocyclic aroyl groups) or O-acyl derivatives of free hydroxyl group (for example that of seryl or threonyl residues) formed with acyl moieties.

The term "derivatives" is intended to include only those derivatives that do not change one amino acid to another of the twenty commonly occurring natural amino acids.

A TRAF-binding protein is a protein or polypeptide, i.e. a sequence of amino acid residues. A polypeptide consisting of a larger sequence which includes the entire sequence of a TRAF-binding protein, in accordance with the definitions herein, is intended to be included within the scope of such a polypeptide as long as the additions do not affect the basic and novel characteristics of the invention, i.e., if they either retain or increase the

biological activity of TRAF-binding protein or can be cleaved to leave a protein or polypeptide having the biological activity of TRAF-binding protein. Thus, for example, the present invention is intended to include fusion proteins of TRAF-binding protein with other amino acids or peptides.

As mentioned above, it should be understood that the above 'TRAF-binding' proteins of the invention are any proteins which may bind and mediate/modulate the activity of any TRAF protein intracellularly. Particular examples are the TRAF2-binding proteins which can modulate or mediate the TRAF2-associated intracellular signaling activity, as mentioned above, especially as concerns TRAF2's involvement in inducing NF-κB activity, in particular, following the interaction between TRAF2 and various members of the TNF/NGF receptor family and/or their associated adaptor proteins as detailed above and below. A specific example of such TRAF2-binding proteins is the NIK protein and its various analogs, fragments, etc. (see Examples) which appears to bind TRAF2 very specifically and to have a direct action in inducing NF-κB activity, with various NIK dominant-negative analogs/muteins blocking this activity.

All the above mentioned modifications are in the scope of the invention provided they preserved the ability of the encoded proteins or polypeptides or their analogs and derivatives thereof, to bind at least the 222-501 amino acid sequence of TRAF2.

All the proteins and polypeptides of the invention by virtue of their capability to bind to TRAF2, are considered as mediators or modulators of TRAF2 signaling. As such, said molecules of the invention have a role in, for example, the signaling process in which the binding of TRAF2 ligand to CD30, CD40, lymphotoxin beta (LT-β) receptor, p55 or p75 TNF receptors, as well as the other receptors and adaptor proteins noted herein above, leads to activation of the transcription factor NF-κB. Particularly interesting is protein NIK and a partial NIK protein, encoded by clone 10 of the invention; a detailed sequence analysis of NIK and this clone-10-encoded protein (originally termed NMPI) disclosed encoded amino acid sequences corresponding to I - XI conserved motifs characteristic to Ser/Thr protein kinases, thus assigning a function to this protein.

The new clones proteins, their analogs, fragments and derivatives have a number of possible uses, for example:

(i) They may be used to mimic or enhance NFκB activity, the function of TRAF2 and the receptors to which they bind, in situations where an enhanced function is desired

such as in anti-tumor or immuno-stimulatory applications where the TRAF2- induced effects are desired. In this case the proteins of the invention, their analogs, fragments or derivatives, which enhance the TRAF2 or receptors effects, may be introduced to the cells by standard procedures known per se. For example, as the proteins encoded by the DNA clones of the invention are intracellular and they should be introduced only into the cells where the TRAF2 effect is desired, a system for specific introduction of these proteins into the cells is necessary. One way of doing this is by creating a recombinant animal virus e.g. one derived from Vaccinia, to the DNA of which the following two genes will be introduced: the gene encoding a ligand that binds to cell surface proteins specifically expressed by the cells e.g. ones such as the AIDs (HIV) virus gp120 protein which binds specifically to some cells (CD4 lymphocytes and related leukemias) or any other ligand that binds specifically to cells carrying a receptor that binds TRAF2, such that the recombinant virus vector will be capable of binding such cells; and the gene encoding the proteins of the invention. Thus, expression of the cell-surface-binding protein on the surface of the virus will target the virus specifically to the tumor cell or other receptor- carrying cell, following which the proteins encoding sequences will be introduced into the cells via the virus, and once expressed in the cells will result in enhancement of the receptor or TRAF2 effect leading to a desired immuno-stimulatory effect in these cells. Construction of such recombinant animal virus is by standard procedures (see for example, Sambrook et al., 1989). Another possibility is to introduce the sequences of the encoded proteins in the form of oligonucleotides which can be absorbed by the cells and expressed therein.

(ii) They may be used to inhibit the NFκB activity, the effects of TRAF2 or of the receptor that binds it, e.g. in cases such as tissue damage as in AIDS, septic shock or graft-vs.-host rejection, in which it is desired to block the induced intracellular signaling. In this situation it is possible, for example, to introduce into the cells, by standard procedures, oligonucleotides having the anti-sense coding sequence for the proteins of the invention, which would effectively block the translation of mRNAs encoding the proteins and thereby block their expression and lead to the inhibition of the undesired effect. Alternatively, other oligonucleotides may be used; oligonucleotides that preserved their ability to bind to TRAF2 in a way that interfers with the binding of other molecules to this protein, while at the same time do not mediate any activation or modulation of this molecule. Having these characteristics, said molecules can disrupt the interaction of TRAF2 with its natural ligand,

therefor acting as inhibitors capable of abolishing effects mediated by TRAF2, such as NF-κB activation, for example. Such oligonucleotides may be introduced into the cells using the above recombinant virus approach, the second sequence carried by the virus being the oligonucleotide sequence.

Another possibility is to use antibodies specific for the proteins of the invention to inhibit their intracellular signaling activity.

Yet another way of inhibiting the undesired effect is by the recently developed ribozyme approach. Ribozymes are catalytic RNA molecules that specifically cleave RNAs. Ribozymes may be engineered to cleave target RNAs of choice, e.g. the mRNAs encoding the proteins of the invention. Such ribozymes would have a sequence specific for the mRNA of the proteins and would be capable of interacting therewith (complementary binding) followed by cleavage of the mRNA, resulting in a decrease (or complete loss) in the expression of the proteins, the level of decreased expression being dependent upon the level of ribozyme expression in the target cell. To introduce ribozymes into the cells of choice (e.g. those carrying the TRAF2 binding proteins) any suitable vector may be used, e.g. plasmid, animal virus (retrovirus) vectors, that are usually used for this purpose (see also (i) above, where the virus has, as second sequence, a cDNA encoding the ribozyme sequence of choice). (For reviews, methods etc. concerning ribozymes see Chen et al., 1992; Zhao and Pick, 1993).

(iii) They may be used to isolate, identify and clone other proteins which are capable of binding to them, e.g. other proteins involved in the intracellular signaling process that are downstream of TRAF2. For example, the DNA sequences encoding the proteins of the invention may be used in the yeast two-hybrid system in which the encoded proteins will be used as "bait" to isolate, clone and identify from cDNA or genomic DNA libraries other sequences ("preys") encoding proteins which can bind to the clones proteins. In the same way, it may also be determined whether the proteins of the present invention can bind to other cellular proteins, e.g. other receptors of the TNF/NGF superfamily of receptors.

(iv) The encoded proteins, their analogs, fragments or derivatives may also be used to isolate, identify and clone other proteins of the same class i.e. those binding to TRAF2 or to functionally related proteins, and involved in the intracellular signaling process. In this application the above noted yeast two-hybrid system may be used, or there may be used a

recently developed system employing non- stringent Southern hybridization followed by PCR cloning (Wilks et al., 1989).

(v) Yet another approach to utilize the encoded proteins of the invention, their analogs, fragments or derivatives is to use them in methods of affinity chromatography to isolate and identify other proteins or factors to which they are capable of binding, e.g., proteins related to TRAF2 or other proteins or factors involved in the intracellular signaling process. In this application, the proteins, their analogs, fragments or derivatives of the present invention, may be individually attached to affinity chromatography matrices and then brought into contact with cell extracts or isolated proteins or factors suspected of being involved in the intracellular signaling process. Following the affinity chromatography procedure, the other proteins or factors which bind to the proteins, their analogs, fragments or derivatives of the invention, can be eluted, isolated and characterized.

(vi) As noted above, the proteins, their analogs, fragments or derivatives of the invention may also be used as immunogens (antigens) to produce specific antibodies thereto. These antibodies may also be used for the purposes of purification of the proteins of the invention either from cell extracts or from transformed cell lines producing them, their analogs or fragments. Further, these antibodies may be used for diagnostic purposes for identifying disorders related to abnormal functioning of the receptor system in which they function, e.g., overactive or underactive TRAF2- induced cellular effects. Thus, should such disorders be related to a malfunctioning intracellular signaling system involving the proteins of the invention, such antibodies would serve as an important diagnostic tool. The term "antibody" is meant to include polyclonal antibodies, monoclonal antibodies (mAbs), chimeric antibodies, anti-idiotypic (anti-Id) antibodies to antibodies that can be labeled in soluble or bound form, as well as fragments thereof, such as, for example, Fab and $F(ab')_2$ - fragments lacking the Fc fragment of intact antibody, which are capable of binding antigen.

(vii) The antibodies, including fragments of antibodies, useful in the present invention may be used to quantitatively or qualitatively detect the clones of the invention in a sample, or to detect presence of cells which express the clones of the present invention. This can be accomplished by immunofluorescence techniques employing a fluorescently labeled antibody coupled with light microscopic, flow cytometric, or fluorometric detection.

The antibodies (or fragments thereof) useful in the present invention may be employed histologically, as in immunofluorescence or immunoelectron microscopy, for *in situ* detection of the clones of the present invention. *In situ* detection may be accomplished by removing a histological specimen from a patient, and providing the labeled antibody of the present invention to such a specimen. The antibody (or fragment) is preferably provided by applying or by overlaying the labeled antibody (or fragment) to a biological sample. Through the use of such a procedure, it is possible to determine not only the presence of the clones, but also its distribution on the examined tissue. Using the present invention, those of ordinary skill will readily perceive that any of wide variety of histological methods (such as staining procedures) can be modified in order to achieve such *in situ* detection.

Such assays for the clones of the present invention typically comprises incubating a biological sample, such as a biological fluid, a tissue extract, freshly harvested cells such as lymphocytes or leukocytes, or cells which have been incubated in tissue culture, in the presence of a detectably labeled antibody capably of identifying the encoded proteins, and detecting the antibody by any of a number of techniques well known in the art.

(viii) The encoded proteins of the invention may also be used as indirect modulators of a number of other proteins by virtue of their capability of binding to other intracellular proteins, which other intracellular proteins directly bind yet other intracellular proteins or an intracellular domain of a transmembrane protein.

For the purposes of modulating these other intracellular proteins or the intracellular domains of transmembranal proteins, the proteins of the invention may be introduced into cells in a number of ways as mentioned hereinabove in (ii).

It should also be noted that the isolation, identification and characterization of the proteins of the invention may be performed using any of the well known standard screening procedures. For example, one of these screening procedures, the yeast two-hybrid procedure which was used to identify the proteins of the invention. Likewise other procedures may be employed such as affinity chromatography, DNA hybridization procedures, etc. as are well known in the art, to isolate, identify and characterize the proteins of the invention or to isolate, identify and characterize additional proteins, factors, receptors, etc. which are capable of binding to the proteins of the invention.

Moreover, the proteins found to bind to the proteins of the invention may themselves be employed, in an analogous fashion to the way in which the proteins of the invention were used as noted above and below, to isolate, identify and characterize other proteins, factors, etc. which are capable of binding to the the proteins of the invention-binding proteins and which may represent factors involved further downstream in the associated signaling process, or which may have signaling activities of their and hence would represent proteins involved in a distinct signaling process.

The DNA sequences and the encoded proteins of the invention may be produced by any standard recombinant DNA procedure (see for example, Sambrook, et al., 1989) in which suitable eukaryotic or prokaryotic host cells are transformed by appropriate eukaryotic or prokaryotic vectors containing the sequences encoding for the proteins. Accordingly, the present invention also concerns such expression vectors and transformed hosts for the production of the proteins of the invention. As mentioned above, these proteins also include their biologically active analogs, fragments and derivatives, and thus the vectors encoding them also include vectors encoding analogs and fragments of these proteins, and the transformed hosts include those producing such analogs and fragments. The derivatives of these proteins are the derivatives produced by standard modification of the proteins or their analogs or fragments, produced by the transformed hosts.

The present invention also relates to pharmaceutical compositions for modulation of the effects mediated by TRAF2. The pharmaceutical compositions comprising, as an active ingredient, any one or more of the following: (i) one or more of the DNA sequences of the invention, or parts of them, subcloned into an appropriate expression vector; (ii) a protein according to the invention, its biologically active fragments, analogs, derivatives or a mixture thereof; (iii) a recombinant animal virus vector encoding for a protein according to the invention, its biologically active fragments, analogs or derivatives.

The pharmaceutical compositions are applied according to the disease to be treated and in amounts beneficial to the patent, depending on body weight and other considerations, as determined by the physician.

As noted above, one of the specific embodiments of the TRAF-binding proteins of the present invention is the TRAF2-binding protein NIK. Based on the findings in accordance with the present invention that NIK binds specifically to TRAF2 and as such is a mediator/modulator of TRAF2 and can thus mediate/modulate TRAF2's activity in NF-

κB activation and hence its possible role in cell survival pathways in ways that TRAF2 functions independently or in conjunction with other proteins (e.g. p55 TNF and p75 TNF receptors, FAS/APO1 receptor, MORT-1, RIP and TRADD) it is of importance to design drugs which may enhance or inhibit the TRAF2-NIK interaction, as desired. For example, when it is desired to increase the cell cytotoxicity induced by TNF it would be desired to inhibit NF-κB induction, by inhibiting the TRAF2-NIK interaction or by inhibiting TRAF2 and/or NIK specifically. Likewise, for example, when it is desired to inhibit the cell cytotoxicity induced by TNF it would be desired to enhance NF-κB induction by enhancing the TRAF2-NIK interaction or by enhancing TRAF2- and/or NIK- specific NF-κB induction. There are many diseases in which such drugs can be of great help. Amongst others, (see above discussion as well) acute hepatitis in which the acute damage to the liver seems to reflect FAS/APO1 receptor-mediated death of the liver cells following induction by the Fas ligand; autoimmune-induced cell death such as the death of the ß Langerhans cells of the pancreas, that results in diabetes; the death of cells in graft rejection (e.g., kidney, heart and liver); the death of oligodendrocytes in the brain in multiple sclerosis; and AIDS-inhibited T cell suicide which causes proliferation of the AIDS virus and hence the AIDS disease.

In such cases, it would be desired to inhibit the FAS/APO1 receptor-mediated cell cytotoxicity (apoptosis) pathway and enhance the FAS/APO1 receptor-mediated induction of NF-κB via TRAF2 and the TRAF2-NIK interaction. One way of doing this would be to increase the amount of NIK in the cells or to increase the amount of TRAF2 and NIK so that the NIK- or TRAF2-NIK- mediated induction of NF-κB activation will be increased providing higher levels of NF-κB activation and hence cell survival; or so that the direct or indirect interaction between FAS/APO1 receptor and TRAF2 (or TRAF2-NIK) will be increased resulting in a decrease in FAS/APO1 receptor interactions with cell cytotoxic mediators (e.g MACH, see scheme in Fig. 2b) to provide for an increase in the induction of NF-κB activation and cell survival.

Conversely, in the case of, for example, tumors and infected cells (see also discussion above) it would be desired to increase the FAS/APO1 receptor-mediated cell cytotoxicity to bring about increased cell death. In this case it would be desired to inhibit FAS/APO1 receptor-TRAF2 (or -TRAF2-NIK) interactions and/or to inhibit NIK directly, and thereby to decrease the induction of NF-κB activity.

It is possible that NIK or one or more of its possible isoforms, analogs or fragments may serve as "natural" inhibitors of NIK itself or of the NIK-TRAF2 interaction, and as such serve as inhibitors of the induction of NF-κB activation. Such inhibitors may thus be employed as the specific inhibitors noted above, for example, those inhibitors to be used when it is desired to increase the cell cytotoxic effects of TNF or the ligand of the FAS/APO1 receptor in order to increase cell death. In fact, as exemplified herein below, various NIK analogs and muteins have been isolated in accordance with the present invention, which are kinase-deficient analogs/muteins and which are capable of blocking the induction of NF-κB activation mediated by the TNF receptors, the FAS/APO1 receptor, their associated proteins TRADD, RIP and MORT1; as well as mediated by the IL-1 receptor (which activation is via NIK but independent of TRAF2); and also as mediated by bacterial endotoxin (LPS), forbol myristate acetate, and the HTLV-1 protein TAX. Likewise, other substances such as peptides, organic compounds, antibodies, etc. may also be screened to obtain specific drugs which are capable of inhibiting the TRAF2-NIK interaction or the activity of NIK.

In a similar fashion, when it is desired to increase the NF-κB activation in various situations as noted above it is possible, for example, to increase the amount of NIK and/or TRAF2 in cells by various standard methods noted herein above (e.g. introducing DNA encoding NIK and/or TRAF2 into cells to induce increased expression, or preparing suitable formulations containing NIK and/or TRAF2 for direct introduction into cells, or any other way known to those of skill in the art). Likewise, other substances such as peptides, organic compounds, etc. may also be screened to obtain specific drugs which are capable of enhancing the activity of NIK or of enhancing the TRAF2-NIK interaction.

A non-limiting example of how peptide inhibitors of the NIK-TRAF2 interaction would be designed and screened is based on previous studies on peptide inhibitors of ICE or ICE-like proteases, the substrate specificity of ICE and strategies for epitope analysis using peptide synthesis. The minimum requirement for efficient cleavage of a peptide by ICE was found to involve four amino acids to the left of the cleavage site with a strong preference for aspartic acid in the P_1 position and with methylamine being sufficient to the right of the P_1 position (Sleath et al., 1990; Howard et al., 1991; Thornberry et al., 1992). Furthermore, the fluorogenic substrate peptide (a tetrapeptide), acetyl-Asp-Glu-Val-Asp-a-(4-methyl-coumaryl-7-amide) abbreviated Ac-DEVD-AMC, corresponds to a sequence in

poly (ADP-ribose) polymerase (PARP) found to be cleaved in cells shortly after FAS-R stimulation, as well as other apoptopic processes (Kaufmann, 1989; Kaufmann et al., 1993; Lazebnik et al., 1994), and is cleaved effectively by CPP32 (a member of the CED3/ICE protease family) and MACH proteases.

As Asp in the P_1 position of the substrate appears to be important, tetrapeptides having Asp as the fourth amino acid residue and various combinations of amino acids in the first three residue positions can be rapidly screened for binding to the active site of the proteases using, for example, the method developed by Geysen (Geysen, 1985; Geysen et al., 1987) where a large number of peptides on solid supports were screened for specific interactions with antibodies. The binding of MACH proteases to specific peptides can be detected by a variety of well known detection methods within the skill of those in the art, such as radiolabeling, etc. This method of Geysen's was shown to be capable of testing at least 4000 peptides each working day.

In a similar way the exact binding region or region of homology which determines the interaction between TRAF2 and NIK (or any other TRAF protein and TRAF-binding protein) can be elucidated and then peptides may be screened which can serve to block this interaction, e.g. peptides synthesized having a sequence similar to that of the binding region or complementary thereto which can compete with natural NIK (or TRAF-binding protein) for binding to TRAF2 (or TRAF).

Since it may be advantageous to design peptide inhibitors that selectively inhibit TRAF2-NIK (or TRAF-TRAF binding protein) interactions without interfering with physiological cell death processes in which other members of the intracellular signaling pathway are involved, e.g. MACH proteases of the cell death pathway, which are members of the CED3/ICE family of proteases, the pool of peptides binding to TRAF2 (or TRAF) or NIK (or TRAF-binding proteins) in an assay such as the one described above can be further synthesized as a fluorogenic substrate peptide to test for selective binding to such other proteins to select only those specific for TRAF2/NIK (or TRAF/TRAF-binding protein). Peptides which are determined to be specific for, for example, TRAF2/NIK, can then be modified to enhance cell permeability and inhibit the activity of TRAF2 and/or NIK either reversibly or irreversibly. Thornberry et al. (1994) reported that a tetrapeptide (acyloxy) methyl ketone Ac-Tyr-Val-Ala-Asp-CH_2OC (O)-[2,6-$(CF_3)_2$] Ph was a potent inactivator of ICE. Similarly, Milligan et al. (1995) reported that tetrapeptide inhibitors having a

chloromethylketone (irreversibly) or aldehyde (reversibly) groups inhibited ICE. In addition, a benzyloxycarboxyl-Asp-CH_2OC (O) -2,6-dichlorobenzene (DCB) was shown to inhibit ICE (Mashima et al., 1995). Accordingly, in an analogous way, tetrapeptides that selectively bind to, for example, TRAF2 or NIK, can be modified with, for example, an aldehyde group, chloromethylketone,(acyloxy) methyl ketone or a CH_2OC (O)-DCB group to create a peptide inhibitor of TRAF2/NIK activity. Further, to improve permeability, peptides can be, for example, chemically modified or derivatized to enhance their permeability across the cell membrane and facilitate the transport of such peptides through the membrane and into the cytoplasm. Muranishi et al. (1991) reported derivatizing thyrotropin-releasing hormone with lauric acid to form a lipophilic lauroyl derivative with good penetration characteristics across cell membranes. Zacharia et al. (1991) also reported the oxidation of methionine to sulfoxide and the replacement of the peptide bond with its ketomethylene isoester ($COCH_2$) to facilitate transport of peptides through the cell membrane. These are just some of the known modifications and derivatives that are well within the skill of those in the art.

Furthermore, drug or peptide inhibitors, which are capable of inhibiting the activity of, for example, NIK by inhibiting the NIK-TRAF2 interaction and likewise, the interaction between TRAF proteins and TRAF-binding proteins can be conjugated or complexed with molecules that facilitate entry into the cell.

U.S. Patent 5,149,782 discloses conjugating a molecule to be transported across the cell membrane with a membrane blending agent such as fusogenic polypeptides, ion-channel forming polypeptides, other membrane polypeptides, and long chain fatty acids, e.g. myristic acid, palmitic acid. These membrane blending agents insert the molecular conjugates into the lipid bilayer of cellular membranes and facilitate their entry into the cytoplasm.

Low et al., U.S. Patent 5, 108,921, reviews available methods for transmembrane delivery of molecules such as, but not limited to, proteins and nucleic acids by the mechanism of receptor mediated endocytotic activity. These receptor systems include those recognizing galactose, mannose, mannose 6-phosphate, transferrin, asialoglycoprotein, transcobalamin (vitamin B_{12}), α-2 macroglobulins, insulin and other peptide growth factors such as epidermal growth factor (EGF). Low et al. teaches that nutrient receptors, such as receptors for biotin and folate, can be advantageously used to enhance transport across the

cell membrane due to the location and multiplicity of biotin and folate receptors on the membrane surfaces of most cells and the associated receptor mediated transmembrane transport processes. Thus, a complex formed between a compound to be delivered into the cytoplasm and a ligand, such as biotin or folate, is contacted with a cell membrane bearing biotin or folate receptors to initiate the receptor mediated trans-membrane transport mechanism and thereby permit entry of the desired compound into the cell.

ICE is known to have the ability to tolerate liberal substitutions in the P_2 position and this tolerance to liberal substitutions was exploited to develop a potent and highly selective affinity label containing a biotin tag (Thornberry et al., 1994). Consequently, the P_2 position as well as possibly the N-terminus of the tetrapeptide inhibitor can be modified or derivatized, such as to with the addition of a biotin molecule, to enhance the permeability of these peptide inhibitors across the cell membrane.

In addition, it is known in the art that fusing a desired peptide sequence with a leader/signal peptide sequence to create a "chimeric peptide" will enable such a "chimeric peptide" to be transported across the cell membrane into the cytoplasm.

As will be appreciated by those of skill in the art of peptides, the peptide inhibitors of the TRAF-TRAF-binding protein interaction, for example, the TRAF2-NIK interaction according to the present invention is meant to include peptidomimetic drugs or inhibitors, which can also be rapidly screened for binding to, for example TRAF2/NIK to design perhaps more stable inhibitors.

It will also be appreciated that the same means for facilitating or enhancing the transport of peptide inhibitors across cell membranes as discussed above are also applicable to the TRAF-binding proteins, for example, NIK, its analogs, fragments or its isoforms themselves as well as other peptides and proteins which exert their effects intracellularly.

As regards the antibodies mentioned herein throughout, the term "antibody" is meant to include polyclonal antibodies, monoclonal antibodies (mAbs), chimeric antibodies, anti-idiotypic (anti-Id) antibodies to antibodies that can be labeled in soluble or bound form, as well as fragments thereof provided by any known technique, such as, but not limited to enzymatic cleavage, peptide synthesis or recombinant techniques.

Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of animals immunized with an antigen. A monoclonal antibody contains a substantially homogeneous population of antibodies specific to antigens, which populations

contains substantially similar epitope binding sites. MAbs may be obtained by methods known to those skilled in the art. See, for example Kohler and Milstein, Nature, 256:495-497 (1975); U.S. Patent No. 4,376,110; Ausubel et al., eds., Harlow and Lane ANTIBODIES : A LABORATORY MANUAL, Cold Spring Harbor Laboratory (1988); and Colligan et al., eds., Current Protocols in Immunology, Greene Publishing Assoc. and Wiley Interscience N.Y., (1992-1996), the contents of which references are incorporated entirely herein by reference. Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, GILD and any subclass thereof. A hybridoma producing a mAb of the present invention may be cultivated *in vitro, in situ* or *in vivo*. Production of high titers of mAbs *in vivo* or *in situ* makes this the presently preferred method of production.

Chimeric antibodies are molecules of which different portions are derived from different animal species, such as those having the variable region derived from a murine mAb and a human immunoglobulin constant region. Chimeric antibodies are primarily used to reduce immunogenicity in application and to increase yields in production, for example, where murine mAbs have higher yields from hybridomas but higher immunogenicity in humans, such that human/murine chimeric mAbs are used. Chimeric antibodies and methods for their production are known in the art (Cabilly et al., *Proc. Natl. Acad. Sci. USA* 81:3273-3277 (1984); Morrison et al., *Proc. Natl. Acad. Sci. USA* 81:6851-6855 (1984); Boulianne et al., *Nature* 312:643-646 (1984); Cabilly et al., European Patent Application 125023 (published November 14, 1984); Neuberger et al., *Nature* 314:268-270 (1985); Taniguchi et al., European Patent Application 171496 (published February 19, 1985); Morrison et al., European Patent Application 173494 (published March 5, 1986); Neuberger et al., PCT Application WO 8601533, (published March 13, 1986); Kudo et al., European Patent Application 184187 (published June 11, 1986); Sahagan et al., *J. Immunol.* 137:1066-1074 (1986); Robinson et al., International Patent Application No. WO8702671 (published May 7, 1987); Liu et al., *Proc. Natl. Acad. Sci USA* 84:3439-3443 (1987); Sun et al., *Proc. Natl. Acad. Sci USA* 84:214-218 (1987); Better et al., *Science* 240:1041-1043 (1988); and Harlow and Lane, ANTIBODIES:A LABORATORY MANUAL, supra. These references are entirely incorporated herein by reference.

An anti-idiotypic (anti-Id) antibody is an antibody which recognizes unique determinants generally associated with the antigen-binding site of an antibody. An Id antibody can be prepared by immunizing an animal of the same species and genetic type

(e.g. mouse strain) as the source of the mAb to which an anti-Id is being prepared. The immunized animal will recognize and respond to the idiotypic determinants of the immunizing antibody by producing an antibody to these idiotypic determinants (the anti-Id antibody). See, for example, U.S. Patent No. 4,699,880, which is herein entirely incorporated by reference.

The anti-Id antibody may also be used as an "immunogen" to induce an immune response in yet another animal, producing a so-called anti-anti-Id antibody. The anti-anti-Id may be epitopically identical to the original mAb which induced the anti-Id. Thus, by using antibodies to the idiotypic determinants of a mAb, it is possible to identify other clones expressing antibodies of identical specificity.

Accordingly, mAbs generated against the TRAF-binding proteins, analogs, fragments or derivatives thereof, (e.g. NIK, its isoforms, analogs, fragments or derivatives) of the present invention may be used to induce anti-Id antibodies in suitable animals, such as BALB/c mice. Spleen cells from such immunized mice are used to produce anti-Id hybridomas secreting anti-Id mAbs. Further, the anti-Id mAbs can be coupled to a carrier such as keyhole limpet hemocyanin (KLH) and used to immunize additional BALB/c mice. Sera from these mice will contain anti-anti-Id antibodies that have the binding properties of the original mAb specific for an epitope of the above TRAF-binding protein, or analogs, fragments and derivatives thereof.

The anti-Id mAbs thus have their own idiotypic epitopes, or "idiotopes" structurally similar to the epitope being evaluated, such as GRB protein-a.

The term "antibody" is also meant to include both intact molecules as well as fragments thereof, such as, for example, Fab and F(ab')2, which are capable of binding antigen. Fab and F(ab')2 fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody (Wahl et al., *J. Nucl. Med.* 24:316-325 (1983)).

It will be appreciated that Fab and F(ab')2 and other fragments of the antibodies useful in the present invention may be used for the detection and quantitation of the TRAF-binding protein according to the methods disclosed herein for intact antibody molecules. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')2 fragments).

An antibody is said to be "capable of binding" a molecule if it is capable of specifically reacting with the molecule to thereby bind the molecule to the antibody. The term "epitope" is meant to refer to that portion of any molecule capable of being bound by an antibody which can also be recognized by that antibody. Epitopes or "antigenic determinants" usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and have specific three dimensional structural characteristics as well as specific charge characteristics.

An "antigen" is a molecule or a portion of a molecule capable of being bound by an antibody which is additionally capable of inducing an animal to produce antibody capable of binding to an epitope of that antigen. An antigen may have one or more than one epitope. The specific reaction referred to above is meant to indicate that the antigen will react, in a highly selective manner, with its corresponding antibody and not with the multitude of other antibodies which may be evoked by other antigens.

The antibodies, including fragments of antibodies, useful in the present invention may be used to quantitatively or qualitatively detect the TRAF-binding protein (e.g. NIK) in a sample or to detect presence of cells which express the TRAF-binding protein of the present invention. This can be accomplished by immunofluorescence techniques employing a fluorescently labeled antibody (see below) coupled with light microscopic, flow cytometric, or fluorometric detection.

The antibodies (or fragments thereof) useful in the present invention may be employed histologically, as in immunofluorescence or immunoelectron microscopy, for *in situ* detection of the TRAF-binding protein of the present invention. *In situ* detection may be accomplished by removing a histological specimen from a patient, and providing the labeled antibody of the present invention to such a specimen. The antibody (or fragment) is preferably provided by applying or by overlaying the labeled antibody (or fragment) to a biological sample. Through the use of such a procedure, it is possible to determine not only the presence of the TRAF-binding protein, but also its distribution on the examined tissue. Using the present invention, those of ordinary skill will readily perceive that any of wide variety of histological methods (such as staining procedures) can be modified in order to achieve such *in situ* detection.

Such assays for the TRAF-binding protein of the present invention typically comprises incubating a biological sample, such as a biological fluid, a tissue extract, freshly

harvested cells such as lymphocytes or leukocytes, or cells which have been incubated in tissue culture, in the presence of a detectably labeled antibody capable of identifying the TRAF-binding protein, and detecting the antibody by any of a number of techniques well known in the art.

The biological sample may be treated with a solid phase support or carrier such as nitrocellulose, or other solid support or carrier which is capable of immobilizing cells, cell particles or soluble proteins. The support or carrier may then be washed with suitable buffers followed by treatment with a detectably labeled antibody in accordance with the present invention, as noted above. The solid phase support or carrier may then be washed with the buffer a second time to remove unbound antibody. The amount of bound label on said solid support or carrier may then be detected by conventional means.

By "solid phase support", "solid phase carrier", "solid support", "solid carrier", "support" or "carrier" is intended any support or carrier capable of binding antigen or antibodies. Well-known supports or carriers, include glass, polystyrene, polypropylene, polyethylene, dextran, nylon amylases, natural and modified celluloses, polyacrylamides, gabbros and magnetite. The nature of the carrier can be either soluble to some extent or insoluble for the purposes of the present invention. The support material may have virtually any possible structural configuration so long as the coupled molecule is capable of binding to an antigen or antibody. Thus, the support or carrier configuration may be spherical, as in a bead, cylindrical, as in the inside surface of a test tube, or the external surface of a rod. Alternatively, the surface may be flat such as a sheet, test strip, etc. Preferred supports or carriers include polystyrene beads. Those skilled in the art will know may other suitable carriers for binding antibody or antigen, or will be able to ascertain the same by use of routine experimentation.

The binding activity of a given lot of antibody, of the invention as noted above, may be determined according to well known methods. Those skilled in the art will be able to determine operative and optimal assay conditions for each determination by employing routine experimentation.

Other such steps as washing, stirring, shaking, filtering and the like may be added to the assays as is customary or necessary for the particular situation.

One of the ways in which an antibody in accordance with the present invention can be detectably labeled is by linking the same to an enzyme and used in an enzyme

immunoassay (EIA). This enzyme, in turn, when later exposed to an appropriate substrate, will react with the substrate in such a manner as to produce a chemical moiety which can be detected, for example, by spectrophotometric, fluorometric or by visual means. Enzymes which can be used to detectably label the antibody include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomeras, yeast alcohol dehydrogenase, alpha-glycerophosphate dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholin-esterase. The detection can be accomplished by colorimetric methods which employ a chromogenic substrate for the enzyme. Detection may also be accomplished by visual comparison of the extent of enzymatic reaction of a substrate in comparison with similarly prepared standards.

Detection may be accomplished using any of a variety of other immunoassays. For example, by radioactive labeling the antibodies or antibody fragments, it is possible to detect R-PTPase through the use of a radioimmunoassay (RIA). A good description of RIA may be found in Laboratory Techniques and Biochemistry in Molecular Biology, by Work, T.S. et al., North Holland Publishing Company, NY (1978) with particular reference to the chapter entitled "An Introduction to Radioimmune Assay and Related Techniques" by Chard, T., incorporated by reference herein. The radioactive isotope can be detected by such means as the use of a g counter or a scintillation counter or by autoradiography.

It is also possible to label an antibody in accordance with the present invention with a fluorescent compound. When the fluorescently labeled antibody is exposed to light of the proper wavelength, its presence can be then detected due to fluorescence. Among the most commonly used fluorescent labeling compounds are fluorescein isothiocyanate, rhodamine, phycoerythrine, pycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine.

The antibody can also be detectably labeled using fluorescence emitting metals such as ^{152}E, or others of the lanthanide series. These metals can be attached to the antibody using such metal chelating groups as diethylenetriamine pentaacetic acid (ETPA).

The antibody can also be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged antibody is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction.

Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

Likewise, a bioluminescent compound may be used to label the antibody of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling are luciferin, luciferase and aequorin.

An antibody molecule of the present invention may be adapted for utilization in an immunometric assay, also known as a "two-site" or "sandwich" assay. In a typical immunometric assay, a quantity of unlabeled antibody (or fragment of antibody) is bound to a solid support or carrier and a quantity of detectably labeled soluble antibody is added to permit detection and/or quantitation of the ternary complex formed between solid-phase antibody, antigen, and labeled antibody.

Typical, and preferred, immunometric assays include "forward" assays in which the antibody bound to the solid phase is first contacted with the sample being tested to extract the antigen from the sample by formation of a binary solid phase antibody-antigen complex. After a suitable incubation period, the solid support or carrier is washed to remove the residue of the fluid sample, including unreacted antigen, if any, and then contacted with the solution containing an unknown quantity of labeled antibody (which functions as a "reporter molecule"). After a second incubation period to permit the labeled antibody to complex with the antigen bound to the solid support or carrier through the unlabeled antibody, the solid support or carrier is washed a second time to remove the unreacted labeled antibody.

In another type of "sandwich" assay, which may also be useful with the antigens of the present invention, the so-called "simultaneous" and "reverse" assays are used. A simultaneous assay involves a single incubation step as the antibody bound to the solid support or carrier and labeled antibody are both added to the sample being tested at the same time. After the incubation is completed, the solid support or carrier is washed to remove the residue of fluid sample and uncomplexed labeled antibody. The presence of

labeled antibody associated with the solid support or carrier is then determined as it would be in a conventional "forward" sandwich assay.

In the "reverse" assay, stepwise addition first of a solution of labeled antibody to the fluid sample followed by the addition of unlabeled antibody bound to a solid support or carrier after a suitable incubation period is utilized. After a second incubation, the solid phase is washed in conventional fashion to free it of the residue of the sample being tested and the solution of unreacted labeled antibody. The determination of labeled antibody associated with a solid support or carrier is then determined as in the "simultaneous" and "forward" assays.

As mentioned above, the present invention also relates to pharmaceutical compositions comprising recombinant animal virus vectors encoding the TRAF-binding proteins, which vector also encodes a virus surface protein capable of binding specific target cell (e.g., cancer cells) surface proteins to direct the insertion of the TRAF-binding protein sequences into the cells. Further pharmaceutical compositions of the invention comprises as the active ingredient (a) an oligonucleotide sequence encoding an anti-sense sequence of the TRAF-binding protein sequence, or (b) drugs that block the TRAF-binding protein- TRAF interaction.

Pharmaceutical compositions according to the present invention include a sufficient amount of the active ingredient to achieve its intended purpose. In addition, the pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically and which can stabilize such preparations for administration to the subject in need thereof as are well known to those of skill in the art.

The TRAF-binding protein and its isoforms or isotypes are suspected to be expressed in different tissues at markedly different levels and apparently also with different patterns of isotypes in an analogous fashion to the expression of various other proteins involved in the intracellular signaling pathways as indicated in the above listed co-owned co-pending patent applications. These differences may possibly contribute to the tissue-specific features of response to the Fas/APO1-ligand and TNF. As in the case of other CED3/ICE homologs (Wang et al., 1994; Alnemri et al., 1995), the present inventors have previously shown (in the above mentioned patent applications) that MACH isoforms that

contain incomplete CED3/ICE regions (e.g., MACHα3) are found to have an inhibitory effect on the activity of co-expressed MACHα1 or MACHα2 molecules; they are also found to block death induction by Fas/APO1 and p55-R. Expression of such inhibitory isoforms in cells may constitute a mechanism of cellular self-protection against Fas/APO1- and TNF-mediated cytotoxicity. The wide heterogeneity of MACH isoforms, which greatly exceeds that observed for any of the other proteases of the CED3/ICE family, should allow a particularly fine tuning of the function of the active MACH isoforms.

In accordance with the present invention there have also been isolated analogs/muteins of one of the TRAF-binding proteins, namely of the TRAF2-binding protein NIK. These NIK analogs/muteins (see above and see Examples below) are inhibitory to NIK-mediated as well as inhibitory to the induction of NF-κB activation mediated by the TNF receptors, FAS/APO1 receptor, their related proteins, the IL-1 receptor and other agents. Hence, as noted above, the TRAF-binding proteins or possible isoforms may have varying effects in different tissues as regards their interaction with TRAF proteins and their influence thereby on the activity of the TRAF proteins, or intracellular signaling mediated by the TRAF proteins.

It is also possible that some of the possible TRAF-binding protein isoforms serve other functions. For example, NIK or some NIK analogs, or isoforms may also act as docking sites for molecules that are involved in other, non-cytotoxic effects of, for example, Fas/APO1 and TNF receptors via interaction with TRAF2 or even independently of TRAF2.

Due to the unique ability of Fas/APO1 and TNF receptors to cause cell death, as well as the ability of the TNF receptors to trigger other tissue-damaging activities, aberrations in the function of these receptors could be particularly deleterious to the organism. Indeed, both excessive and deficient functioning of these receptors have been shown to contribute to pathological manifestations of various diseases (Vassalli, 1992; Nagata and Golstein, 1995). Identifying the molecules that participate in the signaling activity of the receptors, and finding ways to modulate the activity of these molecules, could direct new therapeutic approaches. In view of the suspected important role of TRAF proteins, e.g. TRAF2 and hence the TRAF-TRAF-binding protein, e.g. TRAF2-NIK interaction in Fas/APO1- and TNF-mediated NF-κB activation, it seems particularly important to design drugs that can block the TRAF-TRAF binding protein interaction, e.g.

TRAF2-NIK interaction when it is desired to kill cells (by inhibiting NF-κB activation), and conversely, when it is desired to preserve cells this interaction should be enhanced (to enhance NF-κB activation).

The present invention also concerns proteins or other ligands which can bind to the TRAF-binding proteins of the invention and thereby modulate/mediate the activity of the TRAF-binding proteins. Such proteins or ligands may be screened, isolated and produced by any of the above mentioned methods. For example, there may be isolated a number of new ligands, including proteins, capable of binding to the NIK proteins of the invention (such new proteins/ligands excluding the known TRAF2 and possibly IκB if NIK actually binds I-κB).

As detailed above, such new TRAF-binding protein-binding proteins/ligands, e.g. NIK-binding proteins, may serve as, for example, inhibitors or enhancers of NIK-mediated activity or the activity mediated by the, for example, TRAF2-NIK interaction, and as such will have important roles in various pathological and other situations as detailed above. Another function of such TRAF-binding protein-binding proteins/ligands would be to serve as specific agents for the purification of the TRAF-binding proteins by, for example, affinity chromatography, these new binding proteins/ligands being attached to the suitable chromatography matrices to form the solid or affinity support/matrix through which a solution, extract or the like, containing the TRAF-binding proteins, e.g. NIK, will be passed and in this way to facilitate the purification thereof. Such methods of affinity chromatography are now well known and generally standard procedures of the art.

Likewise, all of the above mentioned TRAF-binding proteins, analogs, fragments, isoforms and derivatives of the present invention may be used to purify by affinity chromatography the various TRAF proteins to which they bind. For example, TRAF2-binding proteins like NIK, and analogs, fragments and muteins of NIK (see examples below) may be used for the affinity chromatography purification of TRAF2. Hence in the same way as the NIK protein, analogs/muteins of the present invention were isolated and produced (see Examples below) using these methods and any other equivalent methods readily apparent to those of skill in the art (as detailed herein above), any other TRAF2-binding proteins may be identified and produced. Such a method for identifying and producing these TRAF-binding proteins, e.g. TRAF2-binding proteins will include a screening step in which the TRAF (e.g. TRAF2) protein, or at least a specific portion

thereof (e.g. the portion of TRAF2 between a.a. 222-501) is used as a substrate or 'bait' to obtain proteins or any other ligand capable of binding thereto; followed by steps of identifying and characterizing such proteins/ligands so-obtained; and subsequently producing such proteins/ligands in substantially isolated and purified forms. All these steps are well known to those of skill in the art and are detailed herein above and herein below.

The invention will now be described in more detail in the following non-limiting examples and the accompanying drawings :

It should also be noted that the procedures of :

i) two-hybrid screen and two-hybrid β-galactosidase expression test; (ii) induced expression, metabolic labeling and immunoprecipitation of proteins; (iii) *in vitro* binding; (iv) assessment of the cytotoxicity; and (v) Northern and sequence analyses, as well as other procedures used in the following Examples have been detailed in previous publications by the present inventors in respect of other intracellular signaling proteins and pathways (see, for example, Boldin et al., 1995a, 1995b, and Boldin et al. 1996). These procedures also appear in detail in the co-owned co-pending Israel Application Nos. 114615, 114986, 115319, 116588, 117932, and 120367 as well as the corresponding PCT application No. PCT/US96/10521). Accordingly, the full disclosures of all these publications and patent applications are included herein in their entirety and at least as far as the detailed experimental procedures are concerned.

EXAMPLES

Materials and Methods

i) cDNA libraries

a) B-cell cDNA library

Oligo dT primed library constructed from human B cells was used (Durfee et al., 1993). The cDNAs of the library were inserted into the XhoI site of the pACT based vector pSE1107 in fusion with GAL4 activation domain.

b) λgt10 testis cDNA library

A cDNA library from human testis was used. The library is a random hexanucleotide primed library with an avarage insert size of 200 to 400 bp.

ii) Yeast strains

Two yeast stains were used as hoststrains for tansformation and screening: HF7c strain that was used in the two hybrid screen and SFY526 strain that was used in the b-galactosidase assays. Both strains carry the auxotrophic markers trp1 and leu2, namely these yeast strains cannot grow in minimal synthetic medium lacking tryptophan and leucine, unless they are transformed by a plasmid carrying the wild-type versions of these genes (TRP1, LEU2). The two yeast strains carry deletion mutations in their GAL4 and GAL80 genes (gal4-542 and gal80-538 mutations, respectively).

SFY526 and HF7c stains carry the lacZ reporter in their genotypes; in SFY526 strain fused to the UAS and the TATA portion of GAL1 promoter, and in HF7c three copies of the GAL4 17-mer consensus sequence and the TATA portion of the CYC1 promoter are fused to lacZ. Both GAL1 UAS and the GAL4 17-mers are responsive to the GAL4 transcriptional activator. In addition, HF7c srain carries the HIS3 reporter fused to the UAS and the TATA portion of GAL1 promoter.

iii) Cloning of human TRAF2

The human TRAF2 was cloned by PCR from an HL60 cDNA library (for TRAF2 sequence and other details see Rothe et al., 1994; Rothe et al., 1995a; Cheng et al., 1996; Hsu et al., 1996; and Wallach, 1996). The primers used were: a) 30-mer forward primer CAGGATCCTCATGGCTGCAGCTAGCGTGAC corresponding to the coding sequence of hTRAF2 starting from the codon for the first methaonine (underlined) and including a linker with BamHI site. b) 32-mer reverse primer GGTCGACTTAGAGCCCTGTCAGGTCCACAATG that includes hTRAF2 gene stop codon (underlined) and a SalI restriction site in its linker. PCR program comprised of an initial denaturation step 2 min. at 94^{0}C followed by 30 cycles of 1 min. at 94^{0}C, 1 min. at 64^{0}C, 1 min. and 40 sec. at 72^{0}C. The amplified human TRAF2 was then inserted into the BamHI - SalI sites of pGBT9 vector in conjunction with GAL 4 DNA Binding domain.

iv) Two hybrid screen of B-cell library

The two hybrid screen is a technique (see details in above mentioned publications and patent applications) used in order to identify factors that are associated with a particular molecule that serves as a "bait". In the present invention TRAF2 that was cloned into the vector pGBT9, served as the bait. TRAF2 was co-expressed together with the screened B-cell cDNA library in the yeast strain HF7c. The PCR-cloned TRAF2 was a

recombinant fusion with the CAL4 DNA- binding domain and the screened cDNA library was fused to the GAL4 activation domain in the pSE1107 vector. The reporter gene in HF7c was HIS3 fused to the upstream activating sequence (UAS) of the GAL1 promoter which is responsive to GAL4 transcriptional activator. Transformants that contained both pGBT9 and pSE1107 plasmids were selected for growth on plates without tryptophan and leucine. In a second step positive clones which expressed two hybrid proteins that interact with each other, and therefore activated GAL1-HIS3, were picked up from plates devoided of tryptophan, leucine and histidine and contained 50 mM 3-aminotriazol (3AT).

v) β-galactosidase assay

Positive clones picked up in the two hybride screen were subjected to lacZ color development test in SFY526 yeast cells, following Clontech Laboratories' manual (for details see above mentioned publications and patent applications). In brief, transformants were allowed to grow at 30^0C for 2-4 days until reaching about 2 mm in diameter, then were transferred onto Whatman filters. The filters went through a freeze/thaw treatment in order to permeabilize the cells, then soaked in a buffer (16.1 mg/ml $Na_2HPO_4 \cdot 7H_2O$; 5.5 mg/ml $NaH_2PO_4 \cdot H_2O$; 0.75 mg/ml KCl; 0.75 mg/ml $MgSO_4 \cdot 7H_2O$, pH=7) containing 0.33 mg/ml X-gal and 0.35 mM β-mercaptoethanol. Colonies were monitored for development of blue color which is an indication for induction of β-galactosidase.

vi) Expression of cloned cDNAs

Two kinds of expression vectors were constructed:

a) A pUHD10-3 based vectors containing the open reading frame (ORF) of either clone 9, 10 or 15 in fusion with the Hemeaglutinine (HA) epitope.

b) A pUHD10-3 based vector into which FLAG octapeptide sequence was introduced just in front of cloned TRAF2, hereby named FLAG/B6/TRAF2.

The constructs containing an ORF of clone 9, 10 or 15 were transfected into HeLa-Bujard cells (for these cells see Gossen, M. and Bujard, M. (1992)) either alone or cotransfected with FLAG/B6/TRAF2 using standard calcium-phosphate method (Method in, for example, Current Protocols in Molecular Biology, eds. Ausubel, F.M et al.)

vii) Luciferase assay

Typically $5x10^5$ transfected cells were harvested by washing three times with cold PBS and resuspending in 400 µl extraction buffer (0.1 M K_2HPO_4/KH_2PO_4 pH=7.8; 1 mM

DTT). Lysis of the cells was achieved by three times freezing in liquid nitrogen and thawing. Cell debris was removed by centrifugation (5 min. at 10,000 x g). For the luciferase assay, 200 µl of luciferase buffer (25 mM glycylglycine, 15 mM K_2HPO_4/KH_2PO_4 pH=7.8, 15 mM $MgSO_4$, 4 mM EGTA, 2 mM ATP, 1 mM DTT) were added to 50 µl of the lysate. Subsequently, 100 µl of 0.2 mM D-luciferine, 25 mM glycylglycine, 1 mM DTT were added to the reaction. Luciferase activity was determined by readind light emission using a Lumitron luminometer set on 10 seconds integration (see above publications and patent applications for additional details).

Example 1: Cloning of new clones 9, 10 and 15

A cDNA library prepared from B-cells was screened for proteins that associate with TRAF2, using the two hybrid technique as described in Materials and Methods (iv). Only in transformants that expressed both TRAF2 and a protein capable of interacting with it, the GAL4 DNA-binding domain and the transcriptional activation domain were brought together. The result was the activation and expression of the reporter gene, in this case HIS3 fused to the UAS and the TATA portion of the GAL1 promoter.

The screen yielded approximately 2000 clones which were able to grow on Trp-, Leu-, His- 3AT plates. DNA prepared from 165 randomly selected positive clones served for transient co-transfection of SFY526 yeast srain together with TRAF2 cloned into pGBT9 vector. Assay for β-galactosidase activity was performed on the transformed SFY526 yeast colonies as described in Materials and Methods (v). The blue color that developed was an indication for yeast colonies that contain cDNA encoding a protein or polypeptide that binds to TRAF2.

The results of the two hybride screen; the ability of the picked clones to grow on 3AT plates and to induce LacZ as measured in the color test, are summarized in Table 1. Of the positive clones checked, two were cDNAs coding for known proteins; TRAF2 itself that is capable of self-associating and forming homodimers, and the lymphotoxin beta receptor whose intacellular domains were shown to bind TRAF2. Three of the cloned cDNAs (clones 9, 10 and 15) were novel.

The positive clones were further checked in a binding specificity test, namely checked for their interaction with irrelevant baits. As shown in Table 2, clones 9 and 10 reacted only with TRAF2 and did not bind to any one of a number of irrelevant proteins

checked. Clone 15, on the other hand, did not bind to MORT1, nor to the intercellular domains of the p55 and p75 TNF receptors, but did weakly bind to Lamin and to Cycline D.

In order to narrow down the region on TRAF2 molecule which interact with clones 9, 10 and 15, two additional constructs were made. One construct comprised of the N-terminal part of the TRAF2 molecule, amino-acids 1 to 221, that included the Ring finger and the zink finger motifs. The second construct included only the C-terminal part of the molecule, amino acids 222 to 501, covering the "TRAF-domain" and additional 42 amino acids. These two constructs were served as baits in two hybrid tests. The results clearly show that while clones 9, 10 and 15 did not interact with the construct comprising amino acids 1 to 221 of TRAF2 molecule, they all did bind to the C-terminal construct comprising the "TRAF domain" with the same efficiency as they bound to the full length TRAF2 molecule.

Table II: **Summary of the results of the two hybrid screen using TRAF2 as a "bait", in which clones 9, 10 and 15 were picked up.**

Growth on 50 mM 3AT	Color test (min.)	ID/name of clone, as defined by its sequencing.	Number of independent clones
+++	10 min	TRAF2	150
++	20 min	new clone number 9	6
+++	15 min	new clone number 10	2
++++	10 min	Lymphotoxin beta receptor	2
+	15 min	new clone number 15	5

Table III : **Specificity tests**

(interaction with irrelevant baits in the two-hybrid test)

clone:	clone 9	clone 10	clone 15
bait			
LAMIN	-	-	+
cyclin D	-	-	+
p75-IC	-	-	-
p55-IC	-	-	-
MORT1	-	-	-
TRAF2	+++	+++	+++

Applying several PCR steps to cDNA clone 10, the full length cDNA was cloned from cDNA libraries obtained from RNA of human tissues. This protein was designated NIK for 'NF-κB inducing kinase' due to the fact that it contains a protein-kinase region (see below). It should be noted that the sequence of clone 10, when intially analyzed (before the obtention of NIK by PCR) was seen to encode for a protein, originally designated NMPI (see co-owned, co-pending IL 117800). This NMPI or clone 10 encoded protein was seen to have sequences corresponding to the I to XI conserved motifs that characterize Ser/Thr protein kinases.

Example 2: Sequencing of new clones

Three of the novel cDNA clones (clones 9, 10 and 15) were purified, amplified in E. Coli and their DNA was subject to sequence analysis. All three clones were found to be partial cDNA clones.

The total lengths of clones 9, 10 and 15 were around 2000, 2700 and 1300 base pairs, respectively.

Figs. 3 and 5 show the sequenced part of clones 9 and 15 and Fig. 4 shows the full sequence of clone 10 :

Figs. 5a-b show the entire nucleotide sequence of clone 15 sequenced from both 5' and 3' ends (a) and the deduced amino acids encoded thereby (b). Clone 15, which is a partial cDNA clone, was found to encode a 172 amino acid long protein.

Clones 9 and 15 are partial clones, which lack their most 5' end of the coding DNA sequences. The deduced amino acid sequences shown in Figs. 3b, 4b and 5b, are all started from the first nucleotide of the respective clone.

The sequence of clone 10 (a partial cDNA clone) which was most thoroughly analyzed, encodes for a protein called NMP1 as noted above, containing Ser/Thr protein kinase motifs. The full length cDNA clone obtained from PCR using the clone 10 as noted above revealed the new TRAF2-binding kinase NIK as mentioned above.

The full nucleotide sequence and its deduced amino acid sequence of NIK are shown in Fig. 6 in which the initiator ATG at nucleotide no. 232 is underlined, and in which the stop codon at nucleotide no. 3073 is indicated by a star. The fully sequenced NIK clone of Fig. 6 is 4596 nucleotides in length within which the NIK coding sequence is contained, this coding for a NIK protein of 947 amino acid residues.

Databank searches revealed that the new amino acid sequence of NIK shows particularly high homology to a group of kinases of which several are known to serve as MAP kinase kinase kinase.

Fig. 7 shows the alignment of :

mouse MEKKK (S1),
BYR2 (S2),
Tpl-2 (S3),
Ewing's sarcoma oncogene (S4),
SS3 (S5),
(STE11) (S6),
(NPK1) (S7),
(BCK1) (S8), and
(NIK) (S9).

Some of those kinases have been identified by virtue of oncogene activity that they possess when in mutated form.

Example 3: Expression of cloned cDNAs and their Co-immunoprecipitation with TRAF2

HeLa-Bujard cells were trasfected with TRAF2 tagged with FLAG in pUHD10-3 based expression vector and constructs containing ORF of either clone 9, 10 or 15 fused to HA epitope, as described in Materials and Methods (iv). Cells were then grown for 24 hrs. in Dulbecco's Modified Eagle's Medium (DMEM) plus 10% calf serum with added ^{35}S-Methionine and ^{35}S-Cysteine. At the end of that incubation time cells were lysed in radioimmune precipitation buffer (10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Nonident P-40, 1% deoxycholate, 0.1% SDS, and 1 mM EDTA; 1 ml/ $5x10^5$ cells), and the lysate was precleared by incubation with irrelevant rabbit antiserum and Protein G-Sepharose beads (Pharmacia, Sweden). Immunoprecipitation was performed by 1 hour incubation at 4°C of aliquots of the lysate with anti-FLAG (purchased from Eastman Kodak Co.) or anti-HA (clone 12CA5 (Field, J. et al. (1988)) monoclonal antibodies. The expressed proteins were analysed on SDS-PAGE gel followed by autoradiography.

The results of such experiments demonstrated that the partial cDNA clones 9, 10 and 15 encoded proteins of molecular weights around 50-65, 45 and 26 kDa respectively.

No interaction of clone 15 with TRAF2 could be detected, but the proteins encoded by clones 9 and 10 (NIK) as well as the full length NIK, were co-immunoprecipitated with the TRAF2 protein. Samples of cells that were co-transfected with TRAF2 and either one of these two clones and immunoprecipitated with either anti-FLAG or anti-HA antibodies followed by analysis on SDS-PAGE as described above, displayed three bands in each lane; one band corresponding to either clone 9 or 10 encoded proteins and the other two is a doublet of 42 and 44 kDa corresponding to TRAF2 protein.

Example 4: Functional tests

NIK was found to have NF-κB induction by gel retardation assay. Typically 0.5-1 x 10^6 293 EBNA cells were transfected with either 10 μg of clone 10 in pcDNA3 (Fig.7 lane 1), 3 μg of pcDNA3 containing cDNA for the p75 TNF receptor (Fig. 7 lane 3), or with both clone 10 (10 μg) and p75 TNF receptor (3 μg) in Fig. 7 lane 2. In each one of the transfections the total amount of transfected DNA was brought to 15 μg with the "empty" pcDNA3 vector. As a control serve 293 EBNA cells transfected with 15 μg pcDNA3 vector alone (Fig. 7 lane 4). Cells were grown for 24 hrs in DMEM medium + 10% calf

serum, then were harvested and treated according to Schreiber et al. (Schreiber, E. et al. (1989). Samples were run on 5 % polyacrylamide gel. NF-κB was monitored using a set of ^{32}P-radiolabelled oligonucleotides corresponding to the NF-κB binding site as probes. (The probes were GATGCCATTGGGGATTTCCTCTTT and CAGTAAAGAGGAAATCCCCAATGG).

As shown in Table IV NIK induced NF-κB even more effectively than TRAF-2. On the other hand, clone 10 did not have this effect at all.

Reporter gene assay was performed as follows :

293 EBNA cells were co-transfected with the pcDNA3 vector containing HIV LTR linked to the luciferase reporter gene, together with either pcDNA3 plasmid containing cDNA for the p75 TNF receptor alone, pcDNA3 plasmid containing clone 10 cDNA alone, or with pcDNA3 plasmid containing cDNA for the p75 TNF receptor and a pcDNA3 plasmid listed in Tables IV and V.

The results shown in Table V demonstrate :

a) that clone 10 transfection does not activate NF-κB induction, while NIK strongly does,

b) that clone 10 as well as NIK in which the active site lysine was replaced with alanine (NIK*) strongly inhibited NF-κB induction by the cDNA listed in the first column of Table IV.

Deletion of the 3' UTR of NIK (NIK-3'UTR) greatly increased its expression and consequently its ability to block NF-κB induction when expressed in the mutated form.

Table IV

Activation of NF-κB by NIK. Gel-retardation assay. Numbers are counts of radioactivity decay events as detected by 'phosphoimager' plate.

transfected cDNA	counts	area (mm^2)
empty vector	327	70.7
TRAF2	3411	70.7
NIK	6532	70.7
clone 10	343	70.7

Table V

Dominant-negative effect of clone 10, NIK K->A mutant on induction of NF-κB by overexpression of TRAF2, TRADD, MORT1/FADD, TNFR-i, TNFR-II, TNFR-I/FAS chimera, RIP and activation of NF-κB by NIK. Luciferase test.

Inducer of NF-κB	empty vector	NIK	NIK-3'UTR	clone 10	NIK*	NIK*-3'UTR	TRAF2 225-501 aa
TRAF2	300	1000		25	30		ND
TRADD	300	800	1000	100	100	5	ND
MORT1/ FADD	300	1000		25	80		90
TNFR-I	200	800	1000	50	100	5	ND
TNFR-II	200	750	800	20	90	6	ND
FAS chimera	300	1200		25	50		30
RIP	300	800		75	50		ND
NIK	500			100		10	ND
TNF	200			80			
RelA	1000	ND	ND	1000	ND	ND	ND

Example 5 : Additional characteristics of NIK

In addition to the specificity tests of Example 2 above, further two-hybrid testing of the binding properties of NIK revealed (results not shown) that the initially isolated partial clone of NIK (NIK 624-947) binds specifically to the C-terminal region of TRAF2 (C-TRAF domain), while, in contrast, the full-length NIK bound to both the C-TRAF domain and a region upstream of it (N-TRAF domain). NIK also does not bind to TRAF3. Further, a chimeric molecule containing the C-TRAF domain of TRAF2 and the N-terminal portion of TRAF3 could bind the partial NIK molecule (NIK 624-947) but not the full-length NIK indicating that the binding of full-length NIK to TRAF2 requires both the C-TRAF and N-TRAF domains of TRAF2.

Moreover, NIK does not self- associate, nor does it bind to the intracellular domains of the : p55 and p75 TNF receptors; the CD40 receptor (a member of the TNF/NGF receptor family); and the FAS/APO1 (CD95 receptor). NIK also does not bind to the intracellular proteins associated with these receptors, such as for example TRADD, MORT1 and RIP. These results correlate with those shown in Table II above concerning the binding specificities of the proteins encoded by clones 9, 10 and 15. The various interactions between the various receptors and proteins are depicted schematically in Figs. 2a and 2b, Fig. 2b being more complete.

Northern blot analysis revealed that there is a single transcript of NIK expressed in various tissues at different levels, which transcript has a size of about 5000 nucleotides which is essentially the same as the cloned NIK cDNA (as noted above, see Fig. 6).

Furthermore, as noted above in respect of the protein encoded by clone 10 (originally designated NMPI), the full-length NIK protein also has a serine/threonine protein kinase motif similar to several MAP kinase kinase kinases (MAPKKK) as also arises from the sequence alignments shown in Fig. 7.

In vitro testing of NIK kinase activity revealed that NIK can be autophosphorylated, but not when the active-site lysine and adjacent lysine are replaced with alanine (NIK analog or mutein designated NIK KK429-430AA indicating that the lysines in positions 429 and 430 are replaced with alanines). This also correlates with the above results set forth in Example 4 and shown in Table IV with respect to the NIK* mutein.

As mentioned above, overexpression of NIK in 293 EBNA cells induced NF-κB to an even greater extent than overexpression of TRAF2, but overexpression of the partial NIK (NIK 624-947) did not bring about NF-κB activation. In addition, the above noted NIK analog/mutein NIK KK429-430AA also did not bring about NF-κB activation when overexpressed in these cells. Thus, induction of NF-κB by NIK depends on an intact kinase function of NIK. In contrast, RIP (see Figs. 2a, b) which also has a kinase domain can still induce NF-κB activation when its kinase activity is abolished by mutation.

The activation of NF-κB upon overexpression of NIK was indistinguishable from that produced by treating the cells with TNF, and as with TNF or TRAF2 overexpression, the principal components of NIK-activated NF-κB were p50 and p65. NIK overexpression caused the degradation of IκBα and blocking this degradation with N-acetyl-Leu-Leu-

norleucinol (ALLN) resulted (as with TNF) in the accumulation of IκB molecules having slower SDS-PAGE migration indicative of phosphorylated IκBα.

Other tests have revealed that NF-κB can be activated in 293-EBNA cells by TNF as well as by overexpression of p55 and p75 TNF receptors, or overexpression of a p55 TNF receptor in which the intracellular domain of the p55 TNF receptor is replaced by that of the FAS/APO1 receptor. NF-κB can also be activated by overexpression of TRAF2, TRADD, RIP or MORT1, but not by a MORT1 deletion mutant lacking the region upstream of the 'death domain' of MORT1. As noted above, full length NIK, but not the NIK mutein NIK KK429-430AA nor the partial NIK (NIK 624-947), induces NF-κB activation. Moreover, expression of the NIK KK429-430AA mutein or NIK 624-947 in 293-EBNA cells together with any of the other above noted agents, i.e. the receptors or associated proteins resulted in the blocking of induction of NF-κB activation by all of these agents, indicating that NIK activity is directly involved in this NF-κB induction. Likewise the above observed inhibition by inactive NIK molecules correlates with less IκB reduction.

NF-κB is also activated by IL-1 (see scheme in Fig. 2b). This effect is apparently independent of TRAF2 (IL-1 does not bind TRAF2 and the IL-1 effect is not blocked by the expression of a TRAF2 dominant-negative mutant). However, this IL-1 effect is inhibited by the expression of NIK mutants. In addition, the NF-κB activity observed upon overexpression of the p65 Rel homologue in 293-EBNA cells was unaffected by co-expression of kinase-deficient NIK mutants, indicating that NIK does not affect the function of Rel proteins directly, but participates in their receptor-induced activation.

The cytotoxic activity of TNF (apparently mediated by MORT1-associated protease MACH - see Fig. 2b) is subject to negative regulation by some NF-κB-inducible genes. The antagonizing consequences of NF-κB-mediated gene induction and MACH activation may explain why TNF itself, as well as IL-1 can induce cellular resistance to TNF cytotoxicity. In line with this, it has also been found in accordance with the present invention that the expression of NIK dominant-negative mutants in 293-EBNA cells significantly increased their susceptibility to killing by TNF, and that overexpression of native (full-length, wild-type) NIK inhibited the killing of the cells by TNF or by overexpression of the p55 TNF receptor (this receptor has an intracellular domain containing a 'death domain' region that when expressed in cells, in the absence of any TNF, can induce on its own cell cytotoxicity

- see above referred-to publications of present inventors and co-owned, co-pending applications).

Example 6 : Further functional tests for NIK biological activity

In accordance with the present invention, it has also been found that expression of NIK dominant-negative mutants could also block the induction of NF-κB activation in 293-EBNA cells by other inducing agents including : (i) the well known bacterial endotoxin, lipopolysaccharide (LPS); (ii) a well known forbol myristate acetate, which is a known protein kinase C activator; and (iii) the HTLV-1 protein TAX.

Furthermore, the expression of dominant-negative mutants of NIK in the 293-EBNA cells has been found to have essentially no effect on the TNF-induced activation of the Jun kinase indicating that NIK acts in a specific and possibly direct manner to enhance the phosphorylation of IκB without affecting the MAP kinases involved in Jun phosphorylation.

In view of all of the above mentioned it arises that the kinase activity of NIK is part of a signaling cascade that is responsible for NF-κB activation and which cascade is common to the two TNF receptors, the FAS/APO1 receptor and the IL-1-receptor. NIK appears to play a specific role in this cascade. The binding of NIK to TRAF2 may serve to enable NIK to be affected by both the TNF receptors and the FAS/APO1 receptor. By analogy to the MAP kinase cascades, NIK may serve as a substrate for a kinase (MAPKKKK) upon being recruited by TRAF2 to the stimulated receptors, so that when NIK is phosphorylated it phosphorylates and activates other kinases (or may induce directly NF-κB activation by direct phosphorylation of IκB). The IL-1-induced NF-κB activation is independent of TRAF2 and hence the activation of NIK by the IL-1-receptor may be mediated by another protein IRAK, a serine/threonine kinase that is recruited to the IL-1 receptor after stimulation (Cao et al., 1996b), and also by TRAF6 which binds IRAK (see Cao et al., 1996a, as well as scheme in Fig. 2b). As noted above, the target of NIK, or of a cascade of kinases activated by it, is likely to be IκB. NIK may also phosphorylate TRAF proteins or regulatory proteins that bind to them for example TANK-I/TRAF (see Cheng and Baltimore, 1996; Rothe et al., 1996) creating docking sites for other proteins.

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CLAIMS:

1. A DNA sequence encoding a protein capable of binding to a tumor necrosis factor receptor-associated factor (TRAF) molecule.

2. A DNA sequence according to claim 1, wherein the TRAF molecule is TRAF2.

3. A DNA sequence according to claim 2, wherein said encoded protein binds to at least the 222-501 amino acid sequence of TRAF2.

4. A DNA sequence according to any one of claims 1 to 3, selected from the group consisting of:

(a) a cDNA sequence of the herein designated clone 9 comprising the nucleotide sequence depicted in Fig 3a.;

(b) a cDNA sequence of the herein designated clone 10 comprising the nucleotide sequence depicted in Fig 4;

(c) a cDNA sequence of the herein designated clone 15 comprising the nucleotide sequence depicted in Fig. 5a;

(d) a fragment of a sequence (a)-(c) which encodes a biologically active protein capable of binding to least the 222-501 amino acid sequence of TRAF2;

(e) a DNA sequence capable of hybridization to a sequence of (a)-(d) under moderately stringent conditions and which encodes a biologically active protein capable of binding to at least the 222-501 amino acid sequence of TRAF2; and

(f) a DNA sequence which is degenerate as a result of the genetic code to the DNA sequences defined in (a)-(e) and which encodes a biologically active protein capable of binding to at least the 222-501 amino acid sequence of TRAF2.

5. A DNA sequence according to any one of claims 1 to 4, selected from the sequences contained in the herein designated cDNA clones 9 and 15.

6. A DNA sequence according to any one of claims 1 to 4, which DNA encodes a protein that also modulates NF-κB activity.

7. A DNA sequence according to claim 6, selected from the sequences contained in the herein designated cDNA clone 10.

8. A DNA sequence according to claim 1 or 6, comprising the DNA sequence encoding the protein NIK (as herein defined).

9. A DNA sequence encoding the protein NIK, isoforms, fragments or analogs thereof, said NIK, isoforms, fragments or analogs thereof being capable of binding to TRAF2 and which is capable of modulating the activity of NF-κB.

10. A DNA sequence according to claim 9, selected from the group consisting of:

a) a cDNA sequence derived from the coding region of a native NIK protein;

b) DNA sequences capable of hybridization to a sequence of (a) under moderately stringent conditions and which encode a biologically active NIK; and

c) DNA sequences which are degenerate as a result of the genetic code to the sequences defined in (a) and (b) and which encode a biologically active NIK protein.

11. A DNA sequence according to claim 9 or 10 comprising at least part of the sequence depicted in Fig. 6 and encoding at least one active NIK protein, isoform, analog or fragment.

12. A DNA sequence according to claim 11 encoding a NIK protein, isoform, analog or fragment having at least part of the amino acid sequence depicted in Fig. 6.

13. A vector comprising a DNA sequence according to any one of claims 1-12.

14. A vector according to claim 13 capable of being expressed in a eukaryotic host cell.

15. A vector according to claim 13 capable of being expressed in a prokaryotic host cell.

16. Transformed eukaryotic or prokaryotic host cells containing a vector according to any one of claims 13-15.

17. A TRAF-binding protein, isoforms, fragments, analogs and derivatives thereof, encoded by a DNA sequence according to any one of claims 1-12, said protein, isoforms, fragments, analogs and derivatives thereof being capable of binding to at least the portion of the TRAF2 protein between amino acids 222-501 of TRAF2.

18. A protein according to claim 17 being the protein encoded by herein designated clone 10.

19. A protein, isoforms, fragments, analogs and derivatives thereof, according to claim 17 being the NIK protein, isoforms, analogs, fragments and derivatives thereof encoded by the DNA sequence according to any one of claims 1-12.

20. A NIK protein, isoforms, analogs, fragments and derivatives thereof according to claim 19, wherein said protein, isoforms, fragments and derivatives have at least part of the amino acid sequence depicted in Fig. 6.

21. A method for producing a protein, isoform, fragment, analog or derivative thereof according to any one of claims 17-19, which comprises growing a transformed host cell according to claim 16 under conditions suitable for the expression of said protein, isoform, fragment, analog or derivative thereof, effecting post-translational modification, as necessary, for obtaining said protein, isoform, fragment, analog or derivative thereof, isolating said expressed protein, isoform, fragment, analog or derivative.

22. Antibodies or active fragments or derivatives thereof, specific for the TRAF-binding protein, isoform, analog, fragment or derivative thereof according to claim 17 or 18; or specific for the NIK protein, isoform, analog, fragment or derivative thereof according to claim 19 or 20.

23. A method for the modulation or mediation in cells of the activity of NF-κB or any other intracellular signaling activity modulated or mediated by TRAF2 or by other molecules to which a protein, isoform, analog, fragment or derivative thereof according to any one of claims 17-20 binds, said method comprising treating said cells by introducing into said cells one or more of said protein, isoform, analog, fragment or derivative thereof in a form suitable for intracellular introduction thereof, or introducing into said cells a DNA sequence encoding said one or more protein, isoform, analog, fragment or derivative thereof in the form of a suitable vector carrying said sequence, said vector being capable of effecting the insertion of said sequence into said cells in a way that said sequence is expressed in said cells.

24. A method according to claim 23, wherein said treating of cells comprises introducing into said cells a DNA sequence encoding said protein, isoform, fragment, analog or derivative in the form of a suitable vector carrying said sequence, said vector being capable of effecting the insertion of said sequence into said cells in a way that said sequence is expressed in said cells.

25. A method according to claim 23 or 24 wherein said treating of said cells is by transfection of said cells with a recombinant animal virus vector comprising the steps of :

(a) constructing a recombinant animal virus vector carrying a sequence encoding a viral surface protein (ligand) that is capable of binding to a specific cell surface receptor on

the surface of said cells to be treated and a second sequence encoding a protein selected from the said protein, isoforms, analogs, fragments and derivatives according to any one of claims 17-20, that when expressed in said cells is capable of modulating/mediating the activity of NF-κB or any other intracellular signaling activity modulated/mediated by TRAF2 or other said molecules; and

(b) infecting said cells with said vector of (a).

26. A method for modulating TRAF2 modulated/mediated effect on cells comprising treating said cells with antibodies or active fragments or derivatives thereof, according to claim 22, said treating being by application of a suitable composition containing said antibodies, active fragments or derivatives thereof to said cells, wherein when the TRAF2-binding protein or portions thereof of said cells are exposed on the extracellular surface, said composition is formulated for extracellular application, and when said TRAF2-binding proteins are intracellular said composition is formulated for intracellular application.

27. A method for modulating the TRAF2 modulated/mediated effect on cells comprising treating said cells with an oligonucleotide sequence encoding an antisense sequence for at least part of the DNA sequence encoding a TRAF2-binding protein according to any one of claims 1-11, said oligonucleotide sequence being capable of blocking the expression of the TRAF2-binding protein.

28. A method according to claim 27 wherein said oligonucleotide sequence is introduced to said cells via a virus of claim 25 wherein said second sequence of said virus encodes said oligonucleotide sequence.

29. A method for modulating the TRAF2 modulated/mediated effect on cells comprising applying the ribozyme procedure in which a vector encoding a ribozyme sequence capable of interacting with a cellular mRNA sequence encoding a TRAF2-binding protein according to any one of claims 17-20, is introduced into said cells in a form that permits expression of said ribozyme sequence in said cells, and wherein when said ribozyme sequence is expressed in said cells it interacts with said cellular mRNA sequence and cleaves said mRNA sequence resulting in the inhibition of expression of said TRAF2-binding protein in said cells.

30. A method for isolating and identifying proteins, according to any one of claims 17-20, capable of binding directly to TRAF2, comprising applying the yeast two-hybrid

procedure in which a sequence encoding said TRAF2 is carried by one hybrid vector and sequence from a cDNA or genomic DNA library is carried by the second hybrid vector, the vectors then being used to transform yeast host cells and the positive transformed cells being isolated, followed by extraction of the said second hybrid vector to obtain a sequence encoding a protein which binds to said TRAF2.

31. A method according to any one of claims 23-30 wherein said protein is NIK or at least one of the NIK isoforms, analogs, fragments and derivatives thereof.

32. A pharmaceutical composition for the modulation of the TRAF2 modulated/mediated effect on cells comprising, as active ingredient at least one TRAF2-binding proteins, according to any one of claims 17-20, its biologically active fragments, analogs, derivatives or mixtures thereof.

33. A pharmaceutical composition for modulating the TRAF2 modulated/mediated effect on cells comprising, as active ingredient, a recombinant animal virus vector encoding a protein capable of binding a cell surface receptor and encoding at least one TRAF2-binding protein, isoform, active fragments or analogs, according to any one of claims 17-20.

34. A pharmaceutical composition for modulating the TRAF2 modulated/mediated effect on cells comprising as active ingredient, an oligonucleotide sequence encoding an anti-sense sequence of the TRAF2-binding protein mRNA sequence according to any one of claims 1-11.

35. A pharmaceutical composition for the prevention or treatment of a pathological condition associated with NF-κB induction or with any other activity mediated by TRAF2 or by other molecules to which a protein according to any one of claims 17-20 binds, said composition comprising an effective amount of a protein encoded by clone 10 or a DNA molecule coding therefor, or a molecule capable of disrupting the interaction of said protein encoded by clone 10 with TRAF2 or any other molecule to which a protein encoded by clone 10 binds.

36. A pharmaceutical composition for the prevention or treatment of a pathological condition associated with NF-κB induction or with any other activity mediated by TRAF2 or by other molecules to which a protein according to any one of claims 17-20 binds, said composition comprising an effective amount of a NIK protein, isoform, fragment, analog or derivative thereof, or a DNA molecule coding therefor, or a molecule capable of disrupting

the interaction of said NIK protein, isoform, fragment, analog or derivative thereof with TRAF2 or any other molecule to which said NIK protein, isoform, fragment, analog or derivative binds.

37. A pharmaceutical composition for the prevention or treatment of a pathological condition associated with NF-κB induction or with any other activity mediated by TRAF2 or by other molecules to which the protein NIK binds, said composition comprising a molecule capable of interfering with the protein kinase activity of NIK.

38. A pharmaceutical composition for the prevention or treatment of a pathological condition associated with NF-κB induction or with any other activity mediated by TRAF2 or by other molecules to which a protein encoded by clone 10 according to claim 18 binds, said composition comprising an effective amount of a protein encoded by clone 10 or a DNA molecule coding therefor, or a molecule capable of disrupting the interaction of said protein encoded by clone 10 with TRAF2 or any other molecule to which said protein encoded by clone 10 binds.

39. A pharmaceutical composition for the prevention or treatment of a pathological condition associated with NF-κB induction or with any other activity mediated by TRAF2 or by other molecules to which a NIK protein, isoform, fragment, analog or derivative according to claim 19 or 20 binds, said composition comprising an effective amount of a NIK protein, isoform, fragment, analog or derivative thereof, or a DNA molecule coding therefor, or a molecule capable of disrupting the interaction of said NIK protein, isoform, fragment, analog or derivative thereof with TRAF2 or any other molecule to which said NIK protein, isoform, fragment, analog or derivative binds.

40. A method for the prevention or treatment of a pathological condition associated with NF-κB induction or with any other activity mediated by TRAF2 or by other molecules to which a protein according to any one of claims 17-20 binds, said method comprising administering to a patient in need an effective amount of a protein or isoform, fragment, analog and derivative thereof or a mixture of any thereof according to any one of claims 17-20, or a DNA molecule coding therefor, or a molecule capable of disrupting the interaction of said protein or isoform, fragment, analog and derivative thereof or a mixture of any thereof according to any one of claims 17-20 with TRAF2 or any other molecule to which said protein or isoform, fragment, analog and derivative thereof or a mixture of any thereof according to any one of claims 17-20 binds.

41. A method according to claim 40 wherein said protein is encoded by clone 10.

42. A method according to claim 40, wherein said protein is NIK.

43. A method for screening of a ligand capable of binding to a protein according to any one of claims 17-20 comprising contacting an affinity chromatography matrix to which said protein is attached with a cell extract whereby the ligand is bound to said matrix, and eluting, isolating and analyzing said ligand.

44. A method for screening of a DNA sequence coding for a ligand capable of binding to a protein according to any one of claims 17-20 comprising applying the yeast two-hybrid procedure in which a sequence encoding said protein is carried by one hybrid vector and sequences from a cDNA or genomic DNA library are carried by the second hybrid vector, transforming yeast host cells with said vectors, isolating the positively transformed cells, and extracting said second hybrid vector to obtain a sequence encoding said ligand.

45. A method for identifying and producing a ligand capable of modulating the cellular activity modulated/mediated by TRAF2 comprising :

a) screening for a ligand capable of binding to a polypeptide comprising at least a portion of TRAF2 having the amino acid residues 222-501 of TRAF2;

b) identifying and characterizing a ligand, other than TRAF2 or portions of a receptor of the TNF/NGF receptor family, found by said screening step to be capable of said binding; and

c) producing said ligand in substantially isolated and purified form.

46. A method for identifying and producing a ligand capable of modulating the cellular activity modulated or mediated by a protein according to any one of claims 17-20 comprising :

a) screening for a ligand capable of binding to a polypeptide comprising at least a portion of the NIK sequence depicted in Fig. 6;

b) identifying and characterizing a ligand, other than TRAF2 or portions of a receptor of the TNF/NGF receptor family, found by said screening step to be capable of said binding; and

c) producing said ligand in substantially isolated and purified form.

47. A method for identifying and producing a ligand capable of modulating the cellular activity modulated/mediated by NIK comprising :

a) screening for a ligand capable of binding to at least a portion of the NIK sequence depicted in Fig. 6;

b) identifying and characterizing a ligand, other than TRAF2 or portions of a receptor of the TNF/NGF receptor family, found by said screening step to be capable of said binding; and

c) producing said ligand in substantially isolated and purified form.

48. A method for identifying and producing a molecule capable of directly or indirectly modulating the cellular activity modulated/mediated by NIK, comprising :

a) screening for a molecule capable of modulating activities modulated/mediated by NIK;

b) identifying and characterizing said molecule; and

c) producing said molecule in substantially isolated and purified form.

49. A method for identifying and producing a molecule capable of directly or indirectly modulating the cellular activity modulated/mediated by a protein according to any one of claims 17-20, comprising :

a) screening for a molecule capable of modulating activities modulated/mediated by a protein according to any one of claims 17-20;

b) identifying and characterizing said molecule; and

c) producing said molecule in substantially isolated and purified form.

ABSTRACT

A DNA sequence encoding a protein capable of binding to a tumor necrosis factor receptor-associated factor (TRAF) molecule, TRAF-binding proteins, their isoforms, analogs, fragments and derivatives encoded by the DNA sequence, their methods for the production of the DNA sequences and proteins, and the uses for the DNA sequence and proteins.

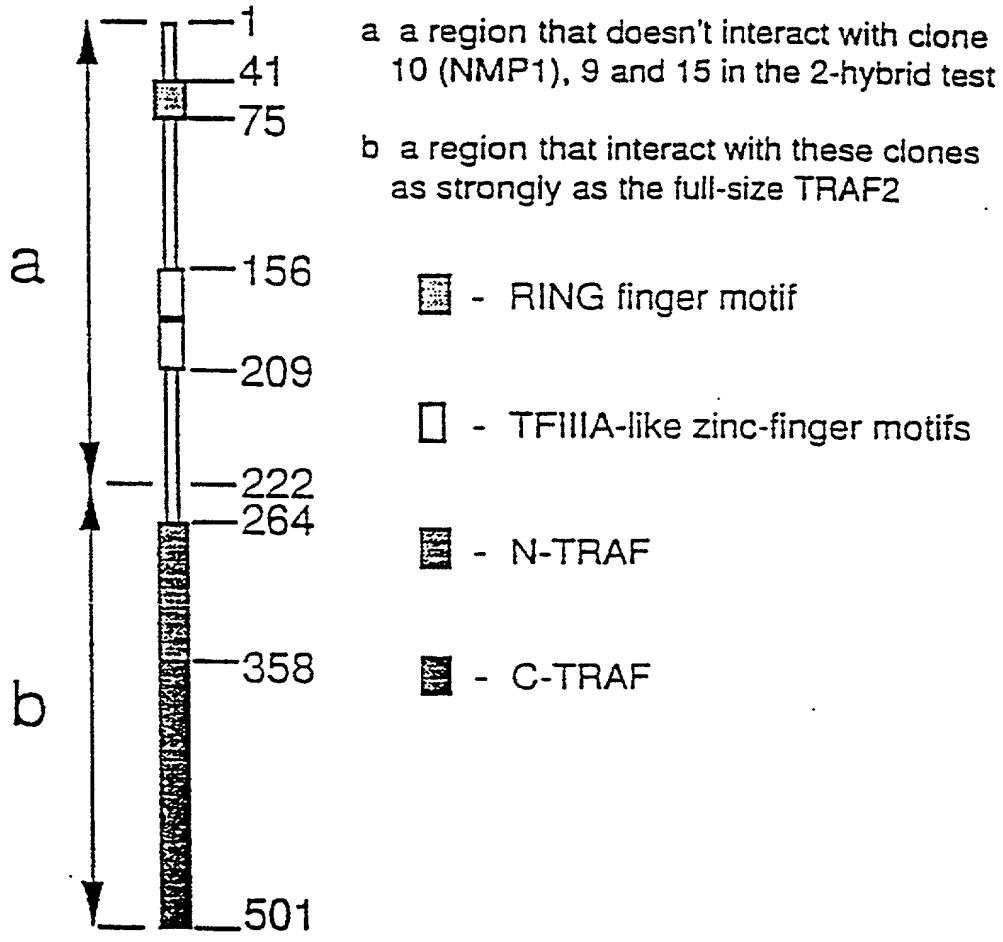
1
41
75
156
209
222
264
358
501
a
b
a a region that doesn't interact with clone 10 (NMP1), 9 and 15 in the 2-hybrid test
b a region that interact with these clones as strongly as the full-size TRAF2
- RING finger motif
- TFIIIA-like zinc-finger motifs
- N-TRAF
- C-TRAF

FIG. 1

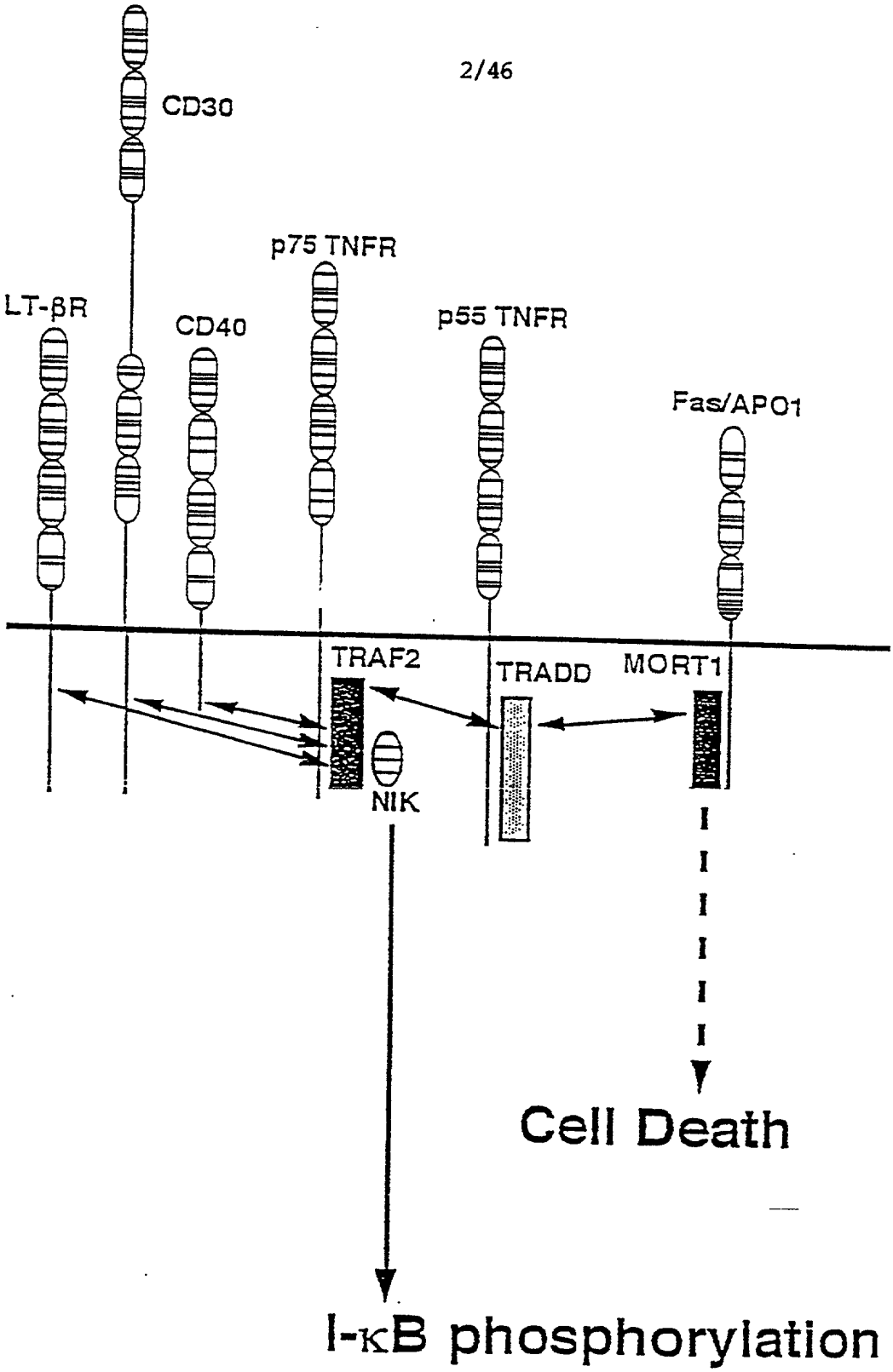
CD30
p75 TNFR
LT-βR
CD40
p55 TNFR
Fas/APO1
TRAF2
TRADD
MORT1
NIK
Cell Death
I-κB phosphorylation

FIG. 2A

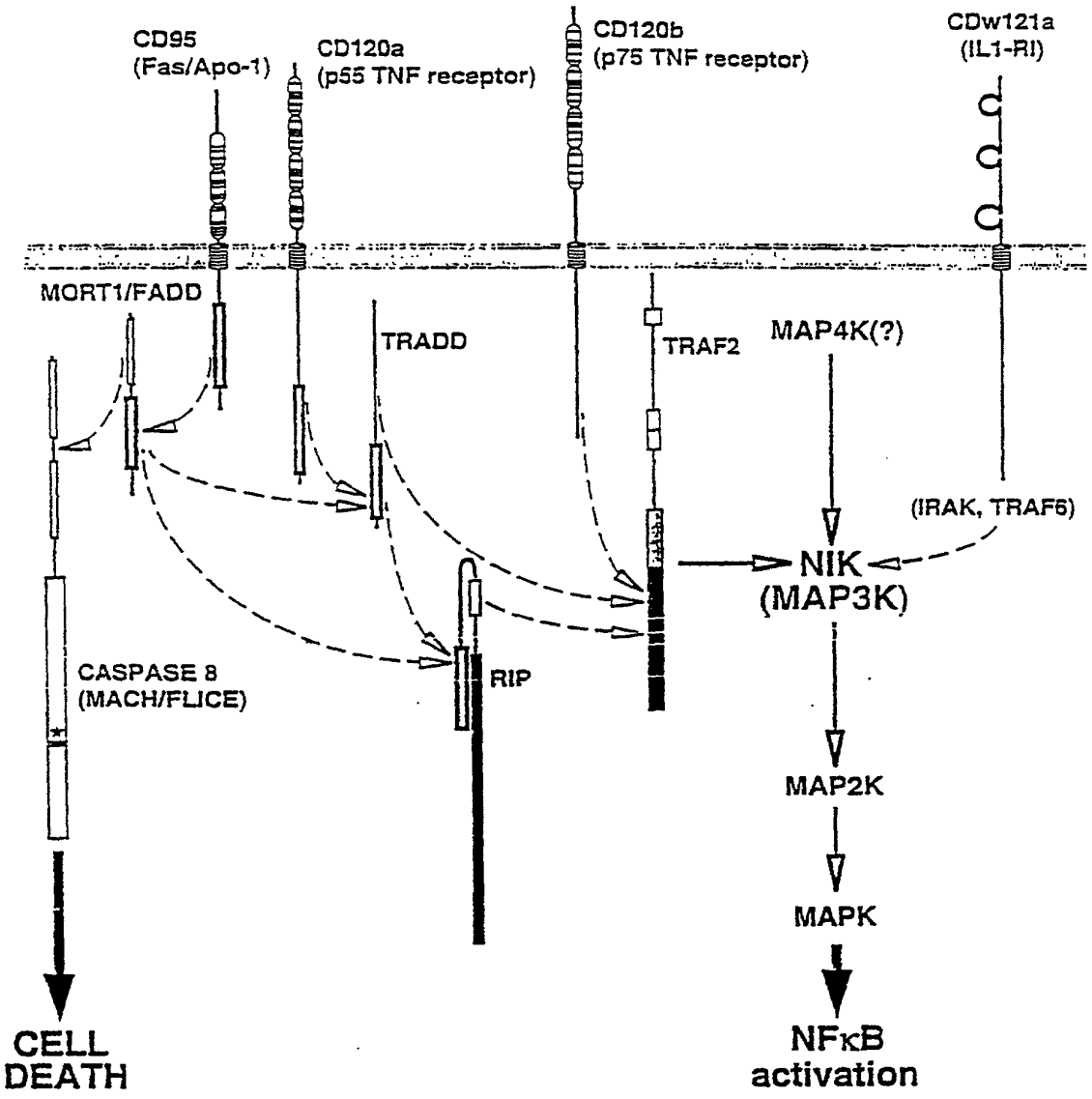
CD95
(Fas/Apo-1)
CD120a
(p55 TNF receptor)
CD120b
(p75 TNF receptor)
CDw121a
(IL1-RI)
MORT1/FADD
TRADD
TRAF2
MAP4K(?)
(IRAK, TRAF6)
NIK
(MAP3K)
CASPASE 8
(MACH/FLICE)
RIP
MAP2K
MAPK
CELL
DEATH
NFκB
activation

FIG. 2B

Length: 1906 July 7, 1996 12:35 Type: N Check: 7122 ..

```
   1  CATTGGGTCA CGCGGTGGCG GCGCTCTAGA ATAGTGGATC CCCCGGGCTG
  51  CAGGAATTCG ATTCGAGGCC ACGAAGGCCG GCGGCGCGGC GCAnGCACCG
 101  GCCCGGGGAn AGGCnCCATG AGCGGATCnC nGAACnATGA CAAAAGACAA
 151  TTTCTGCTGG AGCGACTGCT GGATGCAGTG AAACAGTGCC AGATCCGCTT
 201  TnGAGGGAGA AAGGAGATTG CCTCGGATTC CGACAGCAGG GTCACCTGTC
 251  TGTGTGCCCA GTTTGAAGCC GTCCTGCAGC ATGGCTTGAA GAGGAGTCGA
 301  GGATTGGCAC TCACAGCGGC AGCGATCAAG CAGGCAGCGG GCTTTGCCAG
 351  CAAAACCGAA ACAGAGCCCG TGTTCTGGTA CTACGTGAAG GAGGTCCTCA
 401  ACAAGCACGA GCTGCAGCGC TTCTACTCCC TGCGCCACAT CGCCTCAGAc
 451  gTGGGCCGGG GTCGCGCCTG GCTGcGCTGT GCCCTCAACG AACACTCCCT
 501  GGAGCGCTAC CTGCACATGC TCCTGGCCGA CCGCTGCAGG CTGAGCACTT
 551  TTTATGAAGA CTGGTCTTTT GTGATGGaTG AAGAAAGGTC CAGTATGCTT
 601  CCTACCATGG CAGCAGGTCT GAACTCCATA CTCTTTGCGA TTAACATCGA
 651  CAACAAGGAT TTGAACGGGC AGAGTAAGTT TGCTCCCACC GTTTCAGACC
 701  TCTTAAAGGA GTCAACGCAG AACGTGACCT CCTTGCTGAA GGAGTCCACG
 751  CAAGGAGtGA GCAGCCTGTT CAGGGAGATC ACAGcCTcCT cTGCCGTcTC
 801  CATCcTCATC AAACCTGAAC AGGAGACCGA CCCTTGCCTG TCGTGTCCAG
 851  GAATGTCAGT GCTGATGCCA AATGCAAAAA GGAGCGGAAG AAGAAAAAGA
```

FIG. 3A

```
 901  AAGTGACCAA CATAATCTCA TTTGATGATG AGGAAGATGA GCAGAACTCT
 951  GGGGACGTGT TTAAAAAGAC ACCTGGGGCA GGGGAGAGCT CAGAGGACAA
1001  CTCCGACCGC TCCTCTGTCA ATATCATGTC CGCCTTTGAA AGCCCCTTCG
1051  GGCCTAACTC CAATGGAATC AGAGCAGCAA CTCATGGAAA ATTGATTCCC
1101  TGTCTTTGAA CGGGGAGTTT GGGTACCAGA AGCTTGATGT GAAAAGCATC
1151  GAtGAtGAAG ATgTGGATGA AAACGAAGAT GACgTGTATG GAAACTCATC
1201  AGGAAGGAAG CACAGGGGCC ACTCGGAGTC GCCCGAGAAG CCACTGGAAG
1251  GGAACACCTg CCTCTCCcAG ATGCACAGCT GGgCtCCGCT GAAGgTgCTG
1301  CaCAaTGACT CCGACATCCT CTTCCCTGTC AGTGGCGTGG gCTCCTACAG
1351  CCCAGCAGAT gCCCCCCTCG GAAGCCTGGA GAACGGGACA GGACCAGAGG
1401  ACCACGTTCT CCCGGATCCT GGACTTCGGT ACAGTGTGGA AGCCAGCTCT
```

FIG. 3B

```
1451  CCAGGCCACG GAAGTCCTCT GAGCAGCCTG TTACTTCTGC CTCAGTGCCA
1501  GAGTCCATGA CAATTAGTGA ACTGCGCCAG GCCACTGTGG CCATGATGAA
1551  CAGGAAGGAT GAGCTGGAGG AGGAGAACAG ATCACTGCGA AACCTGCTCG
1601  ACGGTGAGAT GGAGCACTCA GCCGCGCTCC GGCAAGAGGT GGACACCTTG
1651  AAAAGGAAGG TGGCTGAACA GGAGGAGCGG CAGGGCATGA AGGTCCAGGC
1701  GCTGGCCAGC TATCTTTGCT ATTTTGTGAG GAGATTCTAA CCCCACGTGA
1751  GAACCATGTG GTGGAGAAAT GGAGGGAGAG AGAAATCCAA CAGTTCCTGA
1801  TAGTCTCATT TGAGCTCCTG GATCCAGTCT TTCCTGAAGC TGTGTTTCCT
1851  CTGGACTTTT CATGTATGTG AGCCAATAAA TTGCTTTCAT TCCTTGAAAA
1901  AAAAAA
```

FIG. 3C

TRANSLATE of: 9hhh check: 7122 from: 1 to: 1906
generated symbols 1 to: 635.

9hhh.pep Length: 604 August 23, 1996 15:03 Type: P Check: 4554

```
   1  XTGPGXGXMS GSXNXDKRQF LLERLLDAVK QCQIRFXGRK EIASDSDSRV
  51  TCLCAQFEAV LQHGLKRSRG LALTAAAIKQ AAGFASKTET EPVFWYYVKE
 101  VLNKHELQRF YSLRHIASDV GRGRAWLRCA LNEHSLERYL HMLLADRCRL
 151  STFYEDWSFV MDEERSSMLP TMAAGLNSIL FAINIDNKDL NGQSKFAPTV
 201  SDLLKESTQN VTSLLKESTQ GVSSLFREIT ASSAVSILIK PEQETDPCLS
 251  CPGMSVLMPN AKRSGRRKRK *PT*SHLMMR KMSRTLGTCL KRHLGQGRAQ
 301  RTTPTAPLSI SCPPLKAPSG LTPMESEQQL MEN*FPVFER GVWVPEA*CE
 351  KHR**RCG*K RR*RVWKLIR KEAQGPLGVA REATGREHLP LPDAQLGSAE
 401  GAAQ*LRHPL PCQWRGLLQP SRCPPRKPGE RDRTRGPRSP GSWTSVQCGS
 451  QLSRPRKSSE QPVTSASVPE SMTISELRQA TVAMMNRKDE LEEENRSLRN
 501  LLDGEMEHSA ALRQEVDTLK RKVAEQEERQ GMKVQALASY LCYFVRRF*P
 551  HVRTMWWRNG GREKSNSS** SHLSSWIQSF LKLCFLWTFH VCEPINCFHS
 601  LKKK
```

FIG. 3D

clone 10 Length: 2631 August 23, 1996 17:18 Type: N Check: 5107

```
   1 CCCcTcTcAC AGCcCAgGCC ATCCAAGAGG GgCTGAGGAA AGAGCCCATC
  51 cACCGcGTGT cTGCAGcGGA GcTGGGAGGG AAGGTGAACC GGGCAcTACA
 101 GCAAGTGGGA GGTcTGAAGA GCCCTTGGAG GGGAGAATAT AAAGAACCAA
 151 GACATCCACc GCCAAATCAA GCCaAtTACC ACCAGACCcT CcATGCCcAg
 201 CCGAGAGAGc TtTcGCCAAG GGCCCcAGGG CCCCGGCCAg CTGAGGAGAC
 251 AACAggCAGA GCCCcTAAGc TCCAGCcTCC TcTCCCACCA GAGCCCCCAG
 301 AGCCaAACAA GTcTCCTCCC ttGACTttGA GCAAGGAGGA GTcTGGGATG
 351 TGGGAACCCT TACcTctGTC cTCCCTGGAG CCAGCCCCTG CCAGAAACCC
 401 CAGcTCACCA GAGCGGAAAG CAACCGTCCC GGAGCAGGAA CTGCAGCAGC
 451 TGGAAATAGA ATTATTCCTC AACAGCCTGT CCCAGcCATT TTcTcTGGAG
 501 GAGCAGGAGC AAATTCTcTc GTGCCTCAGC ATCGACAGCC TCTCCCtGTC
 551 GGATGACAGT GAGAAGAACC CATCAAAGGC CTCTCAAAGC TCGCGGGACA
 601 CCCTGAGCTC AGGCGTACAC TCCTGGAGCA GcCAGGCCGA GGcTCGAAGc
 651 TCCAGCTGGA ACATGGTGcT GGCCCGGGgg CGgCCCACCG ACACCCCAAG
 701 CTATTTCAAT GGTGTGAAAG TCCAAATACA GTCTCTTAAT GGTGAACACC
 751 TGCACATCCG GGAGTTCCAC CGGGTCAAAG TGGGAGACAT CGCCACTGGC
 801 ATCAGCAGCC AGATCCCAGC TGCAGCCTTC AGCTTGGTCA CCAAAGACGG
 851 GCAGCCTGTT CGCTACGACA TGGAGGTGCC AGACTCGGGC ATCGACCTGC
 901 AGTGCACACT GGCCCCTGAT GGCAGCTTCG CCTGGAGCTG GAGGGTCAAG
 951 CATGGCCAGC TGGAGAACAG GCCCTAACCC TGCCCTCCAC CGCCGGcTCC
1001 ACACTGCCGG aAAGCAGCCT TCCTGCTCGG tGCACGATGC TGCCCTGaAA
1051 AcACAGGcTC AGCcGTTCCC AgGGgATyTG .CCAGcCCCC cGGcTcArcA
1101 G.tGGGaAcC AGGGccTcG. CAGC.AGC.A AGGT.gGGGG CAAGC.AGAA
1151 TGCcTCCCAG GATTTcACA. CcTGAGCCC. TGCCCCA.CC cTGcTGaadA
1201 AAAcAyT.CC GCcAcGtGAA GagAcAGaAG GAGGATGG.C AGGAgTt..A
1251 CcTygGGGAA aCaAAAcAGg gaTcTTt.tT cTGcCCcTGc TCCAGT.cGA
```

FIG. 4A

```
1301  gtTGGCCTG. ACCCGcTTGG A.TCAgtGAC CATTTGtTGG CAGA.CAGGG
1351  GagAgCAGcT TCCAGCcTGG gTCAGAAGGG GTGGGcGAGC CCtTcGGCCC
1401  cTcAcCCT.c cAGGcTGcTG tG.AGAGTGT CAAGTGtGTA AGGG.CCCAA
1451  A.cTcAGG.T TCAGTGCAGA ACCAgGT.CA GCAGGTATGC CCGCCCG.TA
1501  GGTTAA..GG GGGCCcTcT. AAACCCCTTG cCT.GGCCT. CAcCT.GGCC
1551  AGCTCA.CCC cTTTTGGGTG TAGGGGAAAA GAATGCCTGA CCCTGGGAAG
1601  GCTwCCCTGG TAgAATACAC CACACTTTTC AGGTTGTTGC AACACAGGTC
1651  CTGAGTTGAC CTCTGGTTCA GCCAAGGACC AAAGAAGGTG TGTAAGTGAA
1701  GTGGTTCTCA gT.CCCCAgA CATgTgCCCC TTTGCTGCTG GCTACCACTC
1751  TTCCCCAgAg CAGCAGGcCC CgAgCCCCTT CAGGcCCAgC AcTGcCCCAG
1801  AcTCgCTGGC aCTCAGTTCC CTCATCTGTA AAGGTGAAGG GTGATGCAGG

1851  ATATGCCTGA CAGGAACAGT CTGTGGAtGG AcATGATCAg TGcT.AAGG.
1901  AAAGCAGcAG AGaGAGACGy TCcGGCGCCC CAg.CCCCAc T.ATCAGTgT
1951  .CCAgCGTGC T.GGTT.CCC CAg.AGCACA GcT.CAg.CA TcA.CACTGA
2001  CACT.CAcCC T.GCCcTGCC CCT.GGCCA. GAgGGTACTG CCG.ACGGCA
2051  CTTTGCAc.T CTGATG.ACC TCAAAGCACT TTCATGgcT. GcCCTct..G
2101  GCAGGG.CAG GG.CAGGG.C AgTGAcA.CT GTAgG.AGCA TA.gCAA.GC
2151  CAgGAGATGG GGTG.AAGGG A.CACAGTCT TGAGCTGTCC A.CATGCATG
2201  TGAcT.CCTC AAAcCTcTT. .CCAG.ATTT CTCTAAGAAT AGCA.CCCCC
2251  TT.CCCCATT GCCCCAGCTT AgCCTCTTCT CCCAGGGGAG CTA.CTCAgG
2301  ACTCACGTAg CATTAAATCA GCTGTG.AAT CGTCAGGGGG TGTCTGCTAg
2351  CCTCAACCTC CTGGGGCaGG GGACgCCGAg ACTCCGTGGG AgAAgCTCAT
2401  TCcCaCATCT TGCCAAgACA gCCTTT.GTC CAgCTGTCCA CATTGAgTCA
2451  gACTGCTCCC GGGGAgAgAg cCCCGGcCCC CAgCACATAA AGAACTGCAG
2501  CCTTGGTACT GCAGAGTCTG GGTTGTAGAG AACTCTTTGT AAGCAATAAA
2551  GTTTGGGGTG ATGACAAATG TTAAAAAAAG GCCTTCGTGG CCTCGAATCA
2601  AGCTTATCGA TACCGTCGAC CTCGAGGGGG G
```

FIG. 4B

Length: 1253 July 10, 1996 clone15

```
   1  CATTGGAGTC ACGCGGTGGC GGCGCTCTAG AATAGTGGAT CCCCGGGCTg
  51  CA.GGAATTC GATTCGAGcC CACGAAGGCC CCTTCTTCTG TGGTCGCGGC
 101  ACGTTTACaG CCGCAAGCAc CCAGCGGCAg CTGAAGGAGG CTTTTGAgAG
 151  GCTCCTgCCC CAGGTGGAGG CGGCCCGCAA GGCCATCCgC GCCGCTCAGG
 201  TGGAGCGCTA TGTGCCCGAA CACGAGCGAT GCTGCTGGTG CCTGTGCTGC
 251  GGCTGTGAGG TGCGGGAACA CCTGAGCCAT GGAAACCTGA CGGTGCTGTA
 301  CGGGGGgCTG CTGGAGCATC TGGCCAGCCC AGAGCACAAG AAAGCAACCA
 351  ACAAATTCTG GTGGGAGAAC AAAGCTGAGG TCCAGATGAA AGAGAAGTTT
 401  CTGGTCACTC CCCAGGATTA TGCGCGATTC AAGAAATCCA TGGTGAAAGG
 451  TTTGGATTCC TATGAAGAAA AGGAGGATAA AGTGATCAAG GAGaTGgCAG
 501  CTCAGATCCG TGaGGTGGAg CAGAgCCGAC AGGAGgTGGt TCGGtCTGTc
 551  TTAGAgCcTC AGGCAGTGcC AGAcCCAGAA GAGGGcTCTT CAGCAcCTAG
 601  AAGCTGGAAA GGGATGAACA GCCAAGTAGc TTCCAGCTTA CAGcAGcCCT
 651  CAAATTTGGA CCTGCCACCA GCTCCAGAGC TTGAcTGGAT GGAGACAGGA
 701  CCATCTCTGA CATTCATTGG CCATCAGGAT ATACCAGGAG TTGGTAACAT
 751  CCACTCAGGT GCCACACCTC CCTGGATGAT CCAAGATGAA GAATACATTG
 801  CTGGGAACCA AGAAATAGGA CCATCCTATG AAGAATTTCT TAAAGAAAAG
 851  GAAAAACAGA AGTTGAAAAA ACTcCCCCCA GACCGAGTTG GGGCCAACTT
 901  TGATCACAGC TCCAGGACCA GTGCAGGCTG GCTGCCCTCT TTTgGGcCGC
 951  GTCTGGAATA ATGGACGCCG CTGGCAGTCC AGACATCAAC TcCAAAACTG
1001  AAGCTGCAGC AATGAAGAAG CAGTCACATA CAGAAAAAAG CTAATCATGC
1051  TCTCTACCAA CTACCATGAG GCTAAAAGCC AAAGTCAACC AAACCCCTAT
1101  TATACCTTCC ACCCAAATTC TTTATCATTG TCTTTCTTAG GAAACAGACA
1151  TACTCATTCA TTTGATTTAA TAAAGTTTTA TTTTTCGGCC TTCGTGGCCT
1201  CGAATCAAGC TTATCGATAC CGtCGACCTC GAGGGGGGGC CGTACCCACT
1251  TTT
```

FIG. 5A

TRANSLATE of: 15cc check: 9389 from: 2 to: 1253
generated symbols 1 to: 417.

15cc.pep Length: 417 August 23, 1996 14:32 Type: P Check: 7921 ..

```
   1  IGVTRWRRSR IVDPRAAXNS IRAHEGPFFC GRGTFTAAST QRQLKEAFER
  51  LLPQVEAARK AIRAAQVERY VPEHERCCWC LCCGCEVREH LSHGNLTVLY
 101  GGLLEHLASP EHKKATNKFW WENKAEVQMK EKFLVTPQDY ARFKKSMVKG
 151  LDSYEEKEDK VIKEMAAQIR EVEQSRQEVV RSVLEPQAVP DPEEGSSAPR
 201  SWKGMNSQVA SSLQQPSNLD LPPAPELDWM ETGPSLTFIG HQDIPGVGNI
 251  HSGATPPWMI QDEEYIAGNQ EIGPSYEEFL KEKEKQKLKK LPPDRVGANF
 301  DHSSRTSAGW LPSFGPRLE* WTPLAVQTST PKLKLQQ*RS SHIQKKANHA
 351  LYQLP*G*KP KSTKPLLYLP PKFFIIVFLR KQTYSFI*FN KVLFFGLRGL
 401  ESSLSIPSTS RGGRTHF
```

FIG. 5B

```
1
AGC GGG GGG ACT GTG CCG TGT GGA ACG TGT AGC TGT TGA AGG TGG ACT CTG TTA CCA TTG
                                    31
                                    *

61                                  91
AGG ATG TTT GGA GGA TGA GTA TGT GTG GCA GAG GCA CAC ATA AAC AGG CAG AGA CCC TTT
        *

121                                151
GCC CCT GCC TTT CTC CCC CAA CCC AAG GCT GAC CTG TGT TCT CCC AGG TCT GGG ATT CTA

181                                211
AGT GAC CTG CTC TGT GTT TGG TCT CTC TCA GGA TGA GCA CAA GCC TGG GAG ATG GCA GTG
                                    *                       M   A   V

241                                271
ATG GAA ATG GCC TGC CCA GGT GCC CCT GGC TCA GCA GTG GGG CAG CAG AAG GAA CTC CCC
M   E   M   A   C   P   G   A   P   G   S   A   V   G   Q   Q   K   E   L   P

301                                331
AAG CCA AAG GAG AAG ACG CCG CCA CTG GGG AAG AAA CAG AGC TCC GTC TAC AAG CTT GAG
K   P   K   E   K   T   P   P   L   G   K   K   Q   S   S   V   Y   K   L   E

361                                391
GCC GTG GAG AAG AGC CCT GTG TTC TGC GGA AAG TGG GAG ATC CTG AAT GAC GTG ATT ACC
A   V   E   K   S   P   V   F   C   G   K   W   E   I   L   N   D   V   I   T

421                                451
AAG GGC ACA GCC AAG GAA GGC TCC GAG GCA GGG CCA GCT GCC ATC TCT ATC ATC GCC CAG
K   G   T   A   K   E   G   S   E   A   G   P   A   I   S   I   I   A   Q

481                                511
GCT GAG TGT GAG AAT AGC CAA GAG TTC AGC CCC ACC TTT TCA GAA CGC ATT TTC ATC GCT
A   E   C   E   N   S   Q   E   F   S   P   T   F   S   E   R   I   F   I   A

541                                571
GGG TCC AAA CAG TAC AGC CAG TCC GAG AGT CTT GAT CAG ATC CCC AAC AAT GTG GCC CAT
G   S   K   Q   Y   S   Q   S   E   S   L   D   Q   I   P   N   N   V   A   H
```

FIG. 6A

```
601
GCT ACA GAG GGC AAA ATG GCC CGT GTG TGT TGG AAG GGA AAG CGT CGC AGC AAA GCC CGG
A   T   E   G   K   M   A   R   V   C   W   K   G   K   R   R   S   K   A   R
661
AAG AAA CGG AAG AAG AAG AGC TCA AAG TCC CTG GCT CAT GCA GGA GTG GCC TTG GCC AAA
K   K   R   K   K   K   S   S   K   S   L   A   H   A   G   V   A   L   A   K
721
CCC CTC CCC AGG ACC CCT GAG CAG GAG AGC TGC ACC ATC CCA GTG CAG GAG GAT GAG TCT
P   L   P   R   T   P   E   Q   E   S   C   T   I   P   V   Q   E   D   E   S
781
CCA CTC GGC GCC CCA TAT GTT AGA AAC ACC CCG CAG TTC ACC AAG CCT CTG AAG GAA CCA
P   L   G   A   P   Y   V   R   N   T   P   Q   F   T   K   P   L   K   E   P
841
GGC CTT GGG CAA CTC TGT TTT AAG CAG CTT GGC GAG GGC CTA CGG CCG GCT CTG CCT CGA
G   L   G   Q   L   C   F   K   Q   L   G   E   G   L   R   P   A   L   P   R
901
TCA GAA CTC CAC AAA CTG ATC AGC CCC TTG CAA TGT CTG AAC CAC GTG TGG AAA CTG CAC
S   E   L   H   K   L   I   S   P   L   Q   C   L   N   H   V   W   K   L   H
961
CAC CCC CAG GAC GGA GGC CCC CTG CCC CTG CCC ACG CAC CCC TTC CCC TAT AGC AGA CTG
H   P   Q   D   G   G   P   L   P   L   P   T   H   P   F   P   Y   S   R   L
1021
CCT CAT CCC CCC TTC CCA TTC CAC CCT CTC CAG CCC TGG AAA CCT CAC CCT CTG GAG TCC TTC
P   H   P   P   F   F   H   P   L   Q   P   W   K   P   H   P   L   E   S   F
1081
CTG GGC AAA CTG GCC TGT GTA GAC AGC CAG AAA CCC TTG CCT GAC CCA CAC CTG AGC AAA
L   G   K   L   A   C   V   D   S   Q   K   P   L   P   D   P   H   L   S   K
1141
CTG GCC TGT GTA GAC AGT CCA AAG CCC CTG CCT GGC CCA CAC CTG GAG CCC AGC TGC CTG
L   A   C   V   D   S   P   K   P   L   P   G   P   H   L   E   P   S   C   L
1201
TCT CGT GGT GCC CAT GAG AAG TTT TCT GTG GAG GAA TAC CTA GTG CAT GCT CTG CAA GGC
S   R   G   A   H   E   K   F   S   V   E   E   Y   L   V   H   A   L   Q   G
1261
AGC GTG AGC TCA AGC CAG GCC CAC AGC CTG ACC AGC CTG GCC AAG ACC TGG GCA GCA CGG
S   V   S   S   S   Q   A   H   S   L   T   S   L   A   K   T   W   A   A   R
```

FIG. 6B

```
1321                                                1351
GGC TCC AGA TCC CGG GAG CCC AGC CCC AAA ACT GAG GAC AAC GAG GGT GTC CTG CTC ACT
G   S   R   S   R   E   P   S   P   K   T   E   D   N   E   G   V   L   L   T
1381                                                1411
GAG AAA CTC AAG CCA GTG GAT TAT GAG TAC CGA GAA GAA GTC CAC TGG GCC ACG CAC CAG
E   K   L   K   P   V   D   Y   E   Y   R   E   E   V   H   W   A   T   H   Q
1441                                                1471
CTC CGC CTG GGC AGA GGC TCC TTC GGA GAG GTG CAC AGG ATG GAG GAC AAG CAG ACT GGC
L   R   L   G   R   G   S   F   G   E   V   H   R   M   E   D   K   Q   T   G
1501                                                1531
TTC CAG TGC GCT GTC AAA AAG GTG CGC CTG GAA GTA TTT CGG GCA GAG GAG CTG ATG GCA
  F   Q   C   A   V   K   K   K   V   R   L   E   V   F   R   A   E   E   L   M   A

1561                                                1591
TGT GCA GGA TTG ACC TCA CCC AGA ATT GTC CCT TTG TAT GGA GCT GTG AGA GAA GGG CCT
C   A   G   L   T   S   P   R   I   V   P   L   Y   G   A   V   R   E   G   P
1621                                                1651
TGG GTC AAC ATC TTC ATG GAG CTG CTG GAA GGT GGC TCC CTG GGC CAG CTG GTC AAG GAG
W   V   N   I   F   M   E   L   L   E   G   G   S   L   G   Q   L   V   K   E
1681                                                1711
CAG GGC TGT CTC CCA GAG GAC CGG GCC CTG TAC TAC CTG GGC CAG GCC CTG GAG GGT CTG
Q   G   C   L   P   E   D   R   A   L   Y   Y   L   G   Q   A   L   E   G   L
1741                                                1771
GAA TAC CTC CAC TCA CGA AGG ATT CTG CAT GGG GAC GTC AAA GCT GAC AAC GTG CTC CTG
E   Y   L   H   S   R   R   I   L   H   G   D   V   K   A   D   N   V   L   L
1801                                                1831
TCC AGC GAT GGG AGC CAC GCA GCC CTC TGT GAC TTT GGC CAT GCT GTG TGT CTT CAA CCT
S   S   D   G   S   H   A   A   L   C   D   F   G   H   A   V   C   L   Q   P
1861                                                1891
GAT GGC CTG GGA AAG TCC TTG CTC ACA GGG GAC TAC ATC CCT GGC ACA GAG ACC CAC ATG
D   G   L   G   K   S   L   L   T   G   D   Y   I   P   G   T   E   T   H   M
1921                                                1951
GCT CCG GAG GTG GTG CTG GGC AGG AGC TGC GAC GCC AAG GTG GAT GTC TGG AGC AGC TGC
A   P   E   V   V   L   G   R   S   C   D   A   K   V   D   V   W   S   S   C
```

FIG. 6C

```
1981                                                2011
TGT ATG ATG CTG CAC ATG CTC AAC GGC TGC CAC CCC TGG ACT CAG TTC TTC CGA GGG CCG
C   M   M   L   H   M   L   N   G   C   H   P   W   T   Q   F   F   R   G   P
2041                                                2071
CTC TGC CTC AAG ATT GCC AGC GAG CCT CCG CCT GTG AGG GAG ATC CCA CCC TCC TGC GCC
L   C   L   K   I   A   S   E   P   P   P   V   R   E   I   P   P   S   C   A
2101                                                2131
CCT CTC ACA GCC CAG GCC ATC CAA GAG GGG CTG AGG AAA GAG CCC ATC CAC CGC GTG TCT
P   L   T   A   Q   A   I   Q   E   G   L   R   K   E   P   I   H   R   V   S
2161                                                2191
GCA GCG GAG CTG GGA GGG AAG GTG AAC CGG GCA CTA CAG CAA GTG GGA GGT CTG AAG AGC
A   A   E   L   G   G   K   V   N   R   A   L   Q   Q   V   G   G   L   K   S
2221                                                2251
CCT TGG AGG GGA GAA TAT AAA GAA CCA AGA CAT CCA CCG CCA AAT CAA GCC AAT TAC CAC
P   W   R   G   E   Y   K   E   P   R   H   P   P   N   Q   A   N   Y   H
2281                                                2311
CAG ACC CTC CAT GCC CAG CCG AGA GAG CTT TCG CCA AGG GCC CCA GGG CCC CGG CCA GCT
Q   T   L   H   A   Q   P   R   E   L   S   P   R   A   P   G   P   R   P   A
2341                                                2371
GAG GAG ACA ACA GGC AGA GCC CCT AAG CTC CAG CCT CCT CTC CCA CCA GAG CCC CCA GAG
E   E   T   T   G   R   A   P   K   L   Q   P   P   L   P   P   E   P   P   E
2401                                                2431
CCA AAC AAG TCT CCT CCC TTG ACT TTG AGC AAG GAG GAG TCT GGG ATG TGG GAA CCC TTA
P   N   K   S   P   P   L   T   L   S   K   E   E   S   G   M   W   E   P   L
2461                                                2491
CCT CTG TCC TCC CTG GAG CCA GCC CCT GCC AGA AAC CCC AGC TCA CCA GAG CGG AAA GCA
P   L   S   S   L   E   P   A   P   A   R   N   P   S   S   P   E   R   K   A
2521                                                2551
ACC GTC CCG GAG CAG GAA CTG CAG CAG CTG GAA ATA GAA TTA TTC CTC AAC AGC CTG TCC
T   V   P   E   Q   E   L   Q   Q   L   E   I   E   L   F   L   N   S   L   S
2581                                                2611
CAG CCA TTT TCT CTG GAG GAG CAG GAG CAA ATT CTC TCG TGC CTC AGC ATC GAC AGC CTC
Q   P   F   S   L   E   E   Q   E   Q   I   L   S   C   L   S   I   D   S   L
2641                                                2671
TCC CTG TCG GAT GAC AGT GAG AAG AAC CCA TCA AAG GCC TCT CAA AGC TCG CGG GAC ACC
S   L   S   D   D   S   E   K   N   P   S   K   A   S   Q   S   S   R   D   T
2701                                                2731
CTG AGC TCA GGC GTA CAC TCC TGG AGC AGC CAG GCC GAG GCT CGA AGC TCC AGC TGG AAC
L   S   S   G   V   H   S   W   S   S   Q   A   E   A   R   S   S   S   W   N
2761                                                2791
ATG GTG CTG GCC CGG GGG CGG CCC ACC GAC ACC CCA AGC TAT TTC AAT GGT GTG AAA GTC
M   V   L   A   R   G   R   P   T   D   T   P   S   Y   F   N   G   V   K   V
```

FIG. 6D

```
2821                                                2851
CAA ATA CAG TCT CTT AAT GGT GAA CAC CTG CAC ATC CGG GAG TTC CAC CGG GTC AAA GTG
Q   I   Q   S   L   N   G   E   H   L   H   I   R   E   F   H   R   V   K   V
2881                                                2911
GGA GAC ATC GCC ACT GGC ATC AGC AGC CAG ATC CCA GCT GCA GCC TTC AGC TTG GTC ACC
G   D   I   A   T   G   I   S   S   Q   I   P   A   A   A   F   S   L   V   T
2941                                                2971
AAA GAC GGG CAG CCT GTT CGC TAC GAC ATG GAG GTG CCA GAC TCG GGC ATC GAC CTG CAG
K   D   G   Q   P   V   R   Y   D   M   E   V   P   D   S   G   I   D   L   Q
3001                                                3031
TGC ACA CTG GCC CCT GAT GGC AGC TTC GCC TGG AGC TGG AGG GTC AAG CAT GGC CAG CTG
C   T   L   A   P   D   G   S   F   A   W   S   W   R   V   K   H   G   Q   L
3061                                                3091
GAG AAC AGG CCC TAA CCC TGC CCT CCA CCG CCG GCT CCA CAC TGC CGG AAA GCA GCC TTC
E   N   R   P   *

3121                                                3151
CTG CTC GGT GCA CGA TGC TGC CCT GAA AAC ACA GGC TCA GCC GTT CCC AGG GGA TTG CCA

3181                                                3211
GCC CCC CGG CTC ACA GTG GGA ACC AGG GCC TCG CAG CAG CAA GGT GGG GGC AAG CAG AAT

3241                                                3271
GCC TCC CAG GAT TTC ACA CCT GAG CCC TGC CCC ACC CTG CTG AAA AAA CAT CCG CCA CGT

3301                                                3331
GAA GAG ACA GAA GGA TGG CAG GAG TTA CCT GGG GAA ACA AAA CAG GGA TCT TTT TCT

3361                                                3391
GCC CCT GCT CCA GTC GAG TTG GCC TGA CCC GCT TGG ATC AGT GAC CAT TTG TTG GCA GAC

3421                                                3451
AGG GGA GAG CAG CTT CCA GCC TGG GTC AGA AGG GGT GGG CGA GCC CTT CGG CCC CTC ACC
```

Fig. 6E

```
3481                                                3511
CTC CAG GCT GCT GTG AGA GTG TCA AGT GTG TAA GGG CCC AAA CTC AGG TTC AGT GCA GAA

3541                                                3571
CCA GGT CAG CAG GTA TGC CCG CCC GTA GGT TAA GGG GGC CCT CTA AAC CCC TTG CCT GGC

3601                                                3631
CTC ACC TGG CCA GCT CAC CCC TTT TGG GTG TAG GGG AAA AGA ATG CCT GAC CCT GGG AAG

3661                                                3691
GCT CCC TGG TAG AAT ACA CCA CAC TTT TCA GGT TGT TGC AAC ACA GGT CCT GAG TTG ACC

3721                                                3751
TCT GGT TCA GCC AAG GAC CAA AGA AGG TGT GTA AGT GAA GTG GTT CTC AGT CCC CAG ACA

3781                                                3811
TGT GCC CCT TTG CTG CTG GCT ACC ACT CTT CCC CAG AGC AGC AGG CCC CGA GCC CCT TCA

3841                                                3871
GGC CCA GCA CTG CCC CAG ACT CGC TGG CAC TCA GTT CCC TCA TCT GTA AAG GTG AAG GGT

3901                                                3931
GAT GCA GGA TAT GCC TGA CAG GAA CAG TCT GTG GAT GGA CAT GAT CAG TGC TAA GGA AAG

3961                                                3991
CAG CAG AGA GAG ACG TCC GGC GCC CCA GCC CCA CTA TCA GTG TCC AGC GTG CTG GTT CCC

4021                                                4051
CAG AGC ACA GCT CAG CAT CAC ACT GAC ACT CAC CCT GCC CTG CCC CTG GCC AGA GGG TAC

4081                                                4111
TGC CGA CGG CAC TTT GCA CTC TGA TGA CCT CAA AGC ACT TTC ATG GCT GCC CTC TGG CAG
```

FIG. 6F

```
4141                                                4171
GGC AGG GCA GGG CAG TGA CAC TGT AGG AGC ATA GCA AGC CAG GAG ATG GGG TGA AGG GAC

4201                                                4231
ACA GTC TTG AGC TGT CCA CAT GCA TGT GAC TCC TCA AAC CTC TTC CAG ATT TCT CTA AGA

4261                                                4291
ATA GCA CCC CCT TCC CCA TTG CCC CAG CTT AGC CTC TTC TCC CAG GGG AGC TAC TCA GGA

4321                                                4351
CTC ACG TAG CAT TAA ATC AGC TGT GAA TCG TCA GGG GGT GTC TGC TAG CCT CAA CCT CCT

4381                                                4411
GGG GCA GGG GAC GCC GAG ACT CCG TGG GAG AAG CTC ATT CCC ACA TCT TGC CAA GAC AGC

4441                                                4471
CTT TGT CCA GGT GTC CAC ATT GAG TCA GAC TGC TCC CGG GGA GAG AGC CCC GGC CCC CAG

4501                                                4531
CAC ATA AAG AAC TGC AGC CTT GGT ACT GCA GAG TCT GGG TTG TAG AGA ACT CTT TGT AAG

4561                                                4596
CAA TAA AGT TTG GGG TGA TGA CAA ATG TTA AAA AAA
```

FIG. 6G

```
s3   1  --------------------------------- 0
s4   1  --------------------------------- 0
s9   1  --------------------------------- 0
s7   1  --------------------------------- 0
s2   1  --------------------------------- 0
s6   1  --------------------------------- 0
s8   1  MPFLRKIAGTAHTHSRSDSNSSVKFGHQPTSSV 33
s1   1  --------------------------------- 0
s5   1  --------------------------------- 0

s3   1  --------------------------------- 0
s4   1  --------------------------------- 0
s9   1  --------------------------------- 0
s7   1  --------------------------------- 0
s2   1  --------------------------------- 0
s6   1  --------------------------------- 0
s8  34  ASTKSSSKSPRATSRKSIYDDIRSQFPNLTPNS 66
s1   1  --------------------------------- 0
s5   1  --------------------------------- 0
```

FIG. 7A

s3	1	---------------------------------	0
s4	1	---------------------------------	0
s9	1	---------------------------------	0
s7	1	---------------------------------	0
s2	1	---------------------------------	0
s6	1	---------------------------------	0
s8	67	TSSQFYESTPVIEQSFNWTTDDHISAGTLENPT	99
s1	1	---------------------------------	0
s5	1	---------------------------------	0

s3	1	---------------------------------	0
s4	1	---------------------------------	0
s9	1	---------------------------------	0
s7	1	---------------------------------	0
s2	1	---------------------------------	0
s6	1	---------------------------------	0
s8	100	SETNSSYKNDNGPSSLSDSRKSSGGNSVNSLSF	132

FIG. 7B

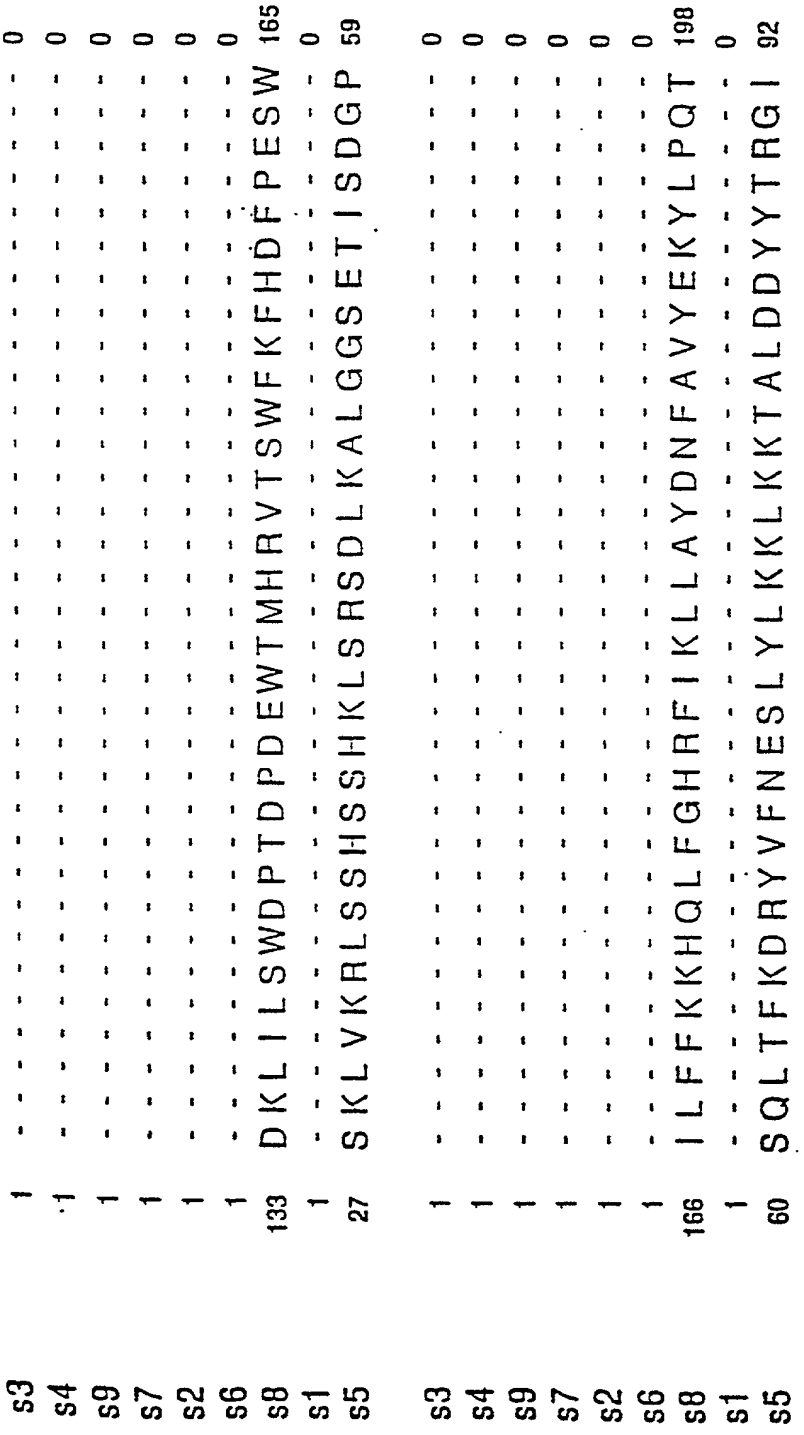
s3 1 ----------D K L I I L S W D P T D P D E W T M H R V T S W F K F H D F P E S W 165
s4 1 ----------- 0
s9 1 ----------- 0
s7 1 ----------- 0
s2 1 ----------- 0
s6 1 ----------- 0
s8 133 ---------- 0
s1 1 ----------- 0
s5 27 ---------- S K L V K R L S S H S S H K L S R S D L K A L G G S E T I S D G P 59
s3 1 ----------- 0
s4 1 ----------- 0
s9 1 ----------- 0
s7 1 ----------- 0
s2 1 ----------- 0
s6 1 ----------- 0
s8 166 ---------- I L F F K K H Q L F G H R F I K L L A Y D N F A V Y E K Y L P Q T 198
s1 1 ----------- 0
s5 60 ---------- S Q L T F K D R Y V F N E S L Y L K K L K K T A L D D Y Y T R G I 92

FIG. 7C

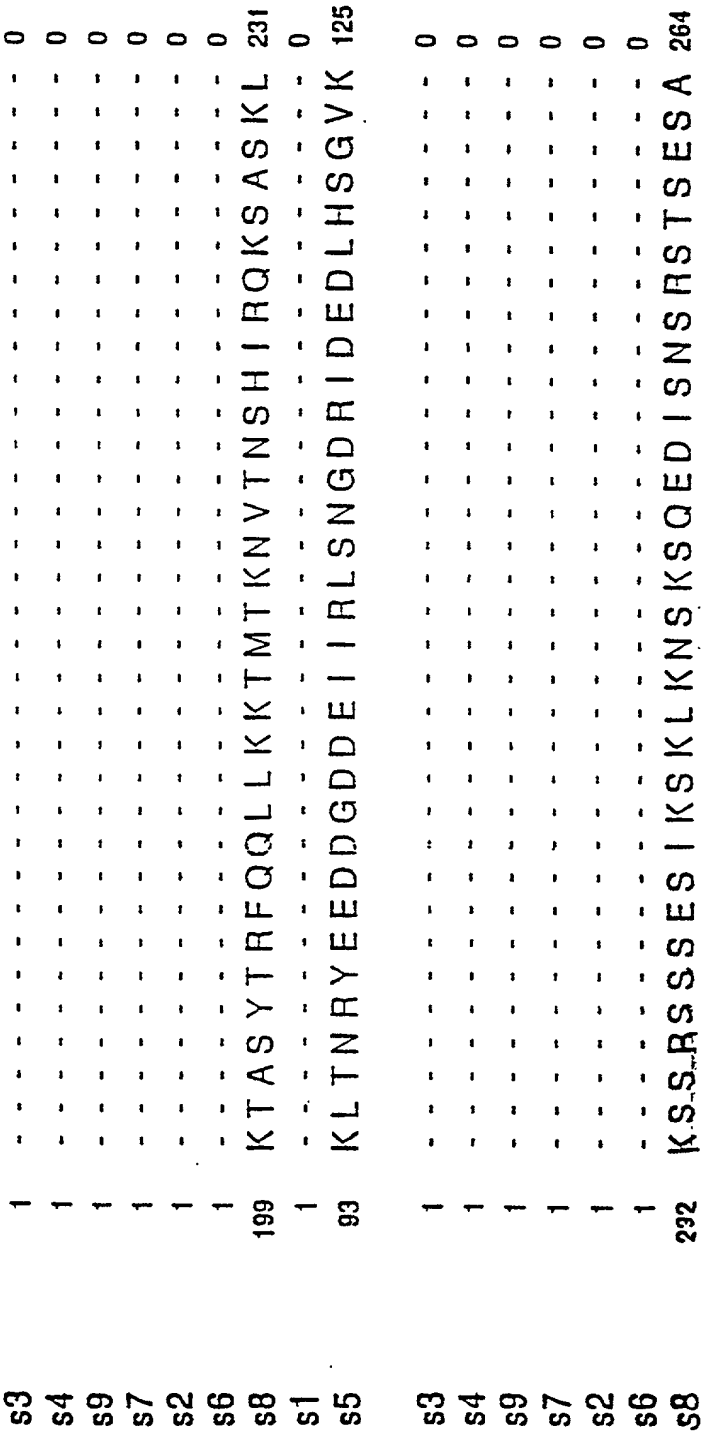
s3 1 ---------- 0
s4 1 ---------- 0
s9 1 ---------- 0
s7 1 ---------- 0
s2 1 ---------- 0
s6 1 ---------- 0
s8 199 KTASYTRFQQLLKKTMTKNVTNSHIRQKSASKL 231
s1 1 ---------- 0
s5 93 KLTNRYEEDDGDDEIIRLSNGDRIDEDLHSGVK 125
s3 1 ---------- 0
s4 1 ---------- 0
s9 1 ---------- 0
s7 1 ---------- 0
s2 1 ---------- 0
s6 1 ---------- 0
s8 232 KSSRSSSESIKSKLKNSKSQEDISNSRSTSESA 264

FIG. 7D

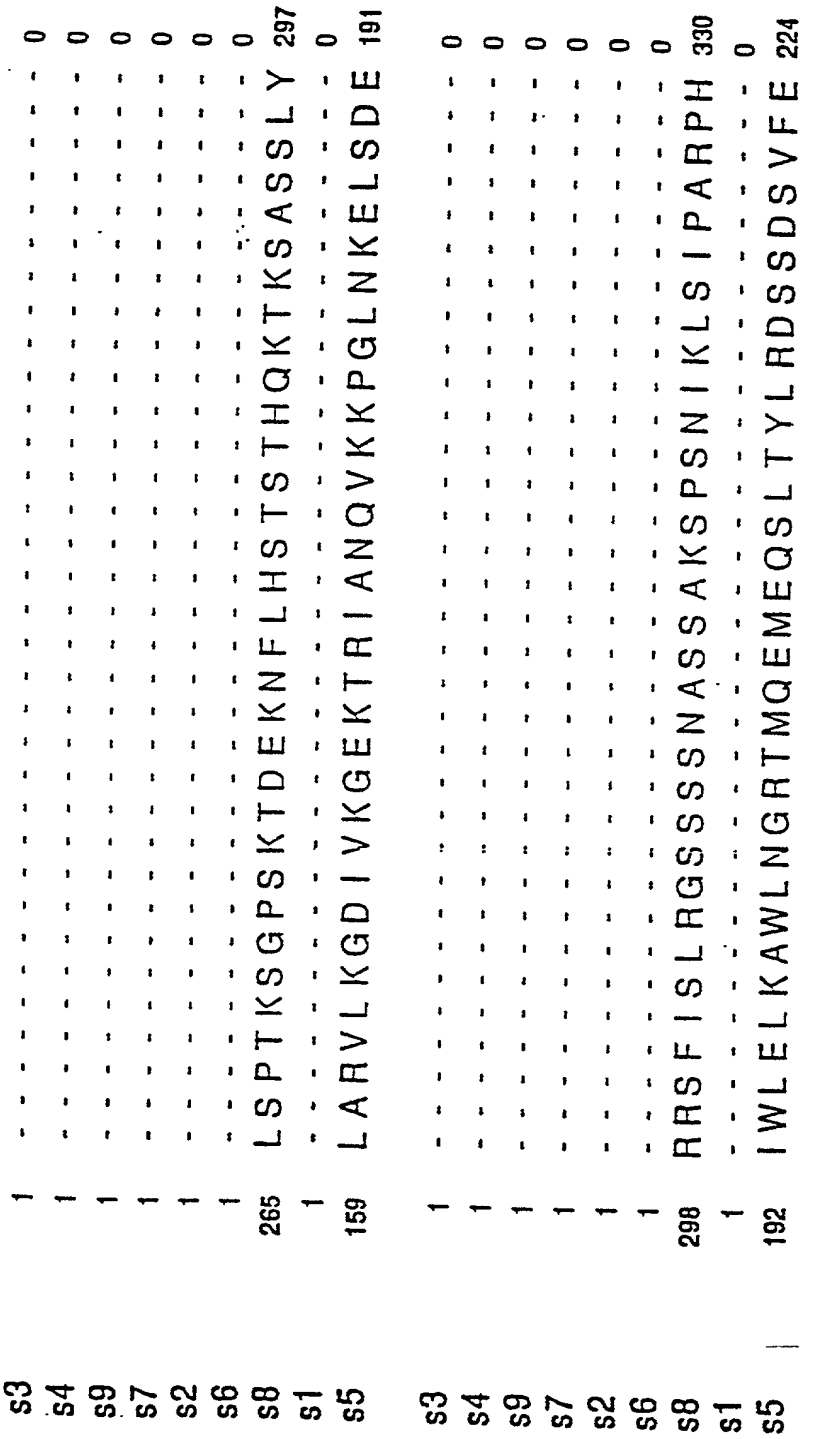
s3 1 ---------- 0
s4 1 ---------- 0
s9 1 ---------- 0
s7 1 ---------- 0
s2 1 ---------- 0
s6 1 ---------- 0
s8 265 LSPTKSGPSKTDEKNFLHSTSTHQKTKSASSLY 297
s1 1 ---------- 0
s5 159 LARVLKGDIVKGEKTRIANQVKKPGLNKELSDE 191
s3 1 ---------- 0
s4 1 ---------- 0
s9 1 ---------- 0
s7 1 ---------- 0
s2 1 ---------- 0
s6 1 ---------- 0
s8 298 RRSFISLRGSSSSNASSAKSPSNIKLSIPARPH 330
s1 1 ---------- 0
s5 192 IWLELKAWLNGRTMQEMEQSLTYLRDSSSDSVFE 224

FIG. 7E

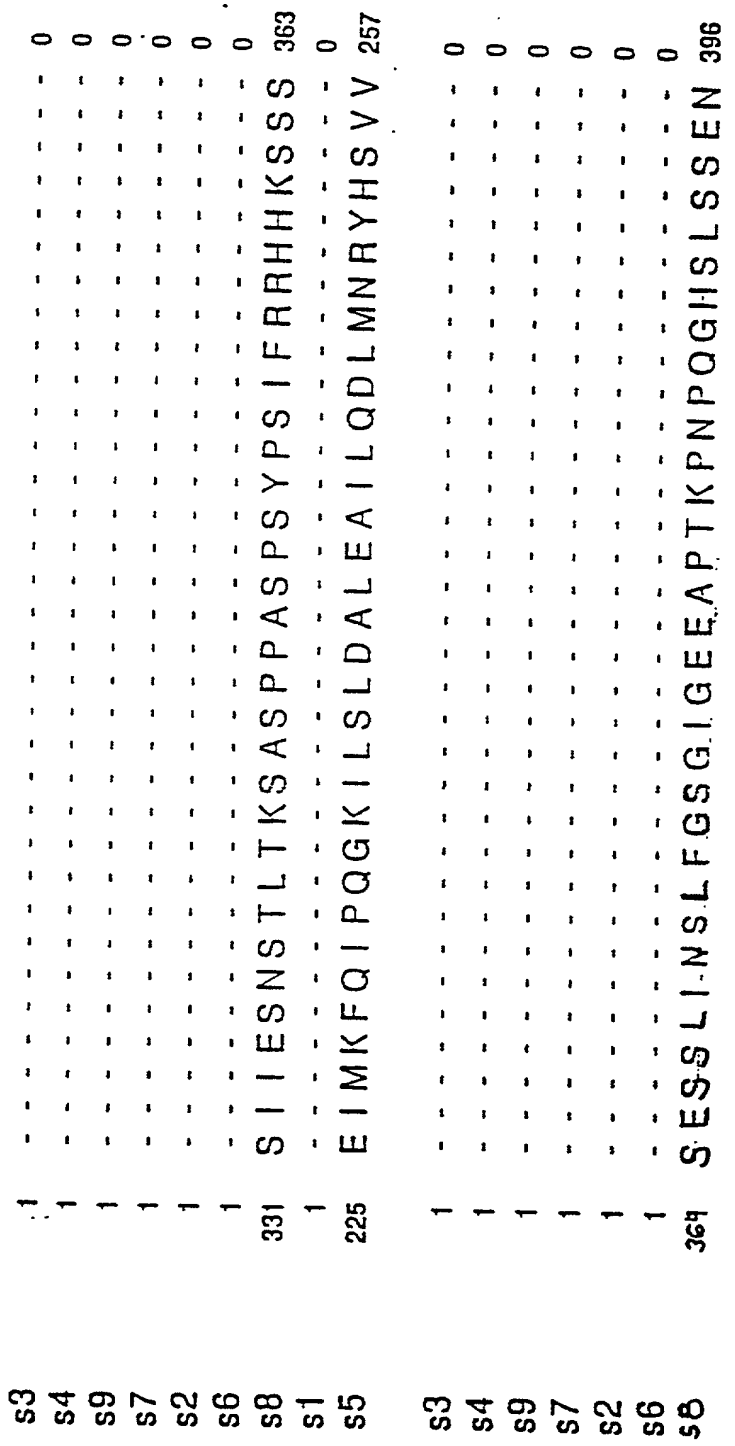
s3 1 ---------- 0
s4 1 ---------- 0
s9 1 ---------- 0
s7 1 ---------- 0
s2 1 ---------- 0
s6 1 ---------- 0
s8 331 SIIESNSTLTKSASPPASPSYPSIFRRHHKSSS 363
s1 1 ---------- 0
s5 225 EIMKFQIPQGKILSLDALEAILQDLMNRYHSVVV 257
s3 1 ---------- 0
s4 1 ---------- 0
s9 1 ---------- 0
s7 1 ---------- 0
s2 1 ---------- 0
s6 1 ---------- 0
s8 361 SESSLI-NSLFGSGIGEEAPTKPNPQGHISLSSEN 396

FIG. 7F

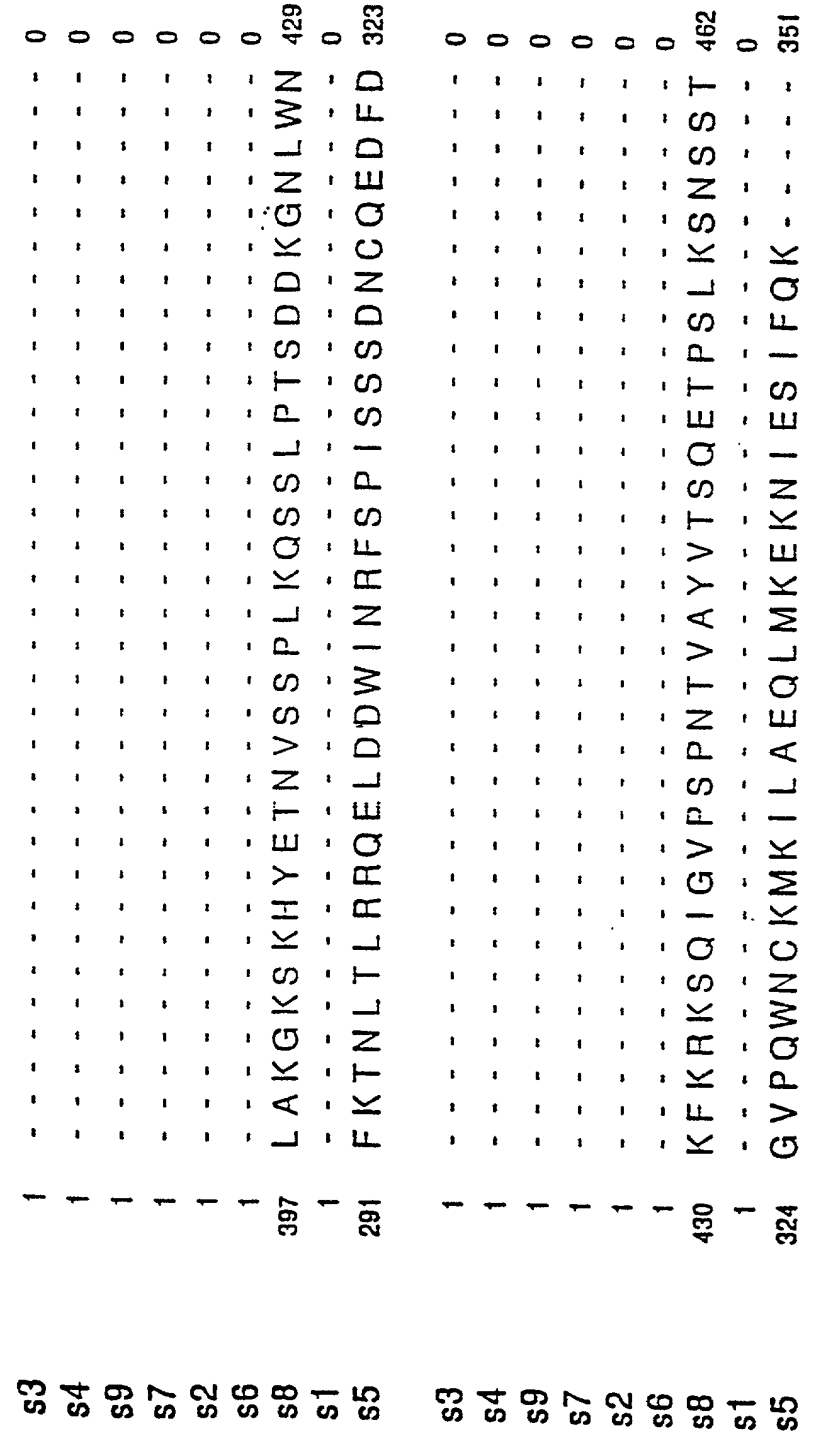
s3 1 ---------- 0
s4 1 ---------- 0
s9 1 ---------- 0
s7 1 ---------- 0
s2 1 ---------- 0
s6 1 ---------- 0
s8 397 LAKGKSKHYETNVSSPLKQSSLPTSDDKGNLWN 429
s1 1 ---------- 0
s5 291 FKTNLTLRRQELDDWINRFSPISSSDNCQEDFD 323
s3 1 ---------- 0
s4 1 ---------- 0
s9 1 ---------- 0
s7 1 ---------- 0
s2 1 ---------- 0
s6 1 ---------- 0
s8 430 KFKRKSQIGVPSPNTVAYYVTSQETPSLKSNSST 462
s1 1 ---------- 0
s5 324 GVPQWNCKMKILAEQLMKEKNIESIFQK---- 351

FIG. 7G

```
s3    1   ---------------------------------  0
s4    1   ---------------------------------  0
s9    1   ---------------------------------  0
s7    1   ---------------------------------  0
s2    1   ---------------------------------  0
s6    1   ---------------------------------  0
s8  463   ATLTVQTADVNIPSPSSSPPPIPKTANRSLEVI  495
s1    1   ---------------------------------  0
s5  352   ----------KIFYPLSPWMFKLKLHFIVYRET  374

s3    1   ---------------------------------  0
s4    1   ---------------------------------  0
s9    1   ---------------------------------  0
s7    1   ---------------------------------  0
s2    1   ---------------------------------  0
s6    1   ---------------------------------  0
s8  496   STEDTPKISSTTASFKETYPDCINPDKTVPVPV  528
```

FIG. 7H

```
s3     1   ---------------------------------   0
s4     1   ---------------------------------   0
s9     1   ---------------------------------   0
s7     1   ---------------------------------   0
s2     1   -----------------------------MEYY   4
s6     1   -------------------MEQTQTAEGTDLLI   14
s8   529   NNQKYSVKNFLLDQKFYPLKKTGLNDSENKYIL   561
s1     1   ---------------------------------   0
s5   408   YARKLKNPTMMMIDQMIDDFNAFIRLSVQLKYT   440

s3     1   ---------------------------------   0
s4     1   ---------------------------------   0
s9     1   ---------------------------------   0
s7     1   ---------------------------------   0
s2     5   TSK---E-------VAEWLKSIG-LEKYIEQFS   26
s6    15   GD---EKTNDLPFVQLFLEEIG-CTQYLDSFIQ   43
s8   562   VTK---D---NVSFVPLNLKSVAKLSSFKESAL   588
s1     1   ---------------------------------   0
s5   441   LTKYCSNLPFDVDFDPTFENTVIEAIRYLFFLL   473
```

FIG. 7I

```
s3    1 ----------------------------------------------------------- 0
s4    1 ----------------------------------------------------------- 0
s9    1 ----------------------------------------------------------- 0
s7    1 ----------------------------------------------------------- 0
s2   27 QNN-IEGRHLNHLTLPLLKDL---------------GIENTA 52
s6   44 CNLVTEEEIKYLDKDILIAL---------------GVNKIGD 70
s8  589 TKLGINHKNVTFHMTDFDCDI---------------GAAIPD 615
s1    1 ----------------------------------------------------------- 0
s5  474 NLKLIDSSKQNFKAPDLLLKYWDHLKNTGHYIN 506

s3    1 ----------------------------------------------------------- 0
s4    1 ----------------------------------------------------------- 0
s9    1 ----------------------------------------------------------- 0
s7    1 ----------------------------------------------------------- 0
s2   53 -KGKQFLK----------------------QRDYLRE---- 66
s6   71 RLKILRK----------------------SKSFQRDKRIEQ 89
s8  616 DTLEFLKKSLFLNTSGKIYI,KDQMKLQQKPKPA 648
```

FIG. 7J

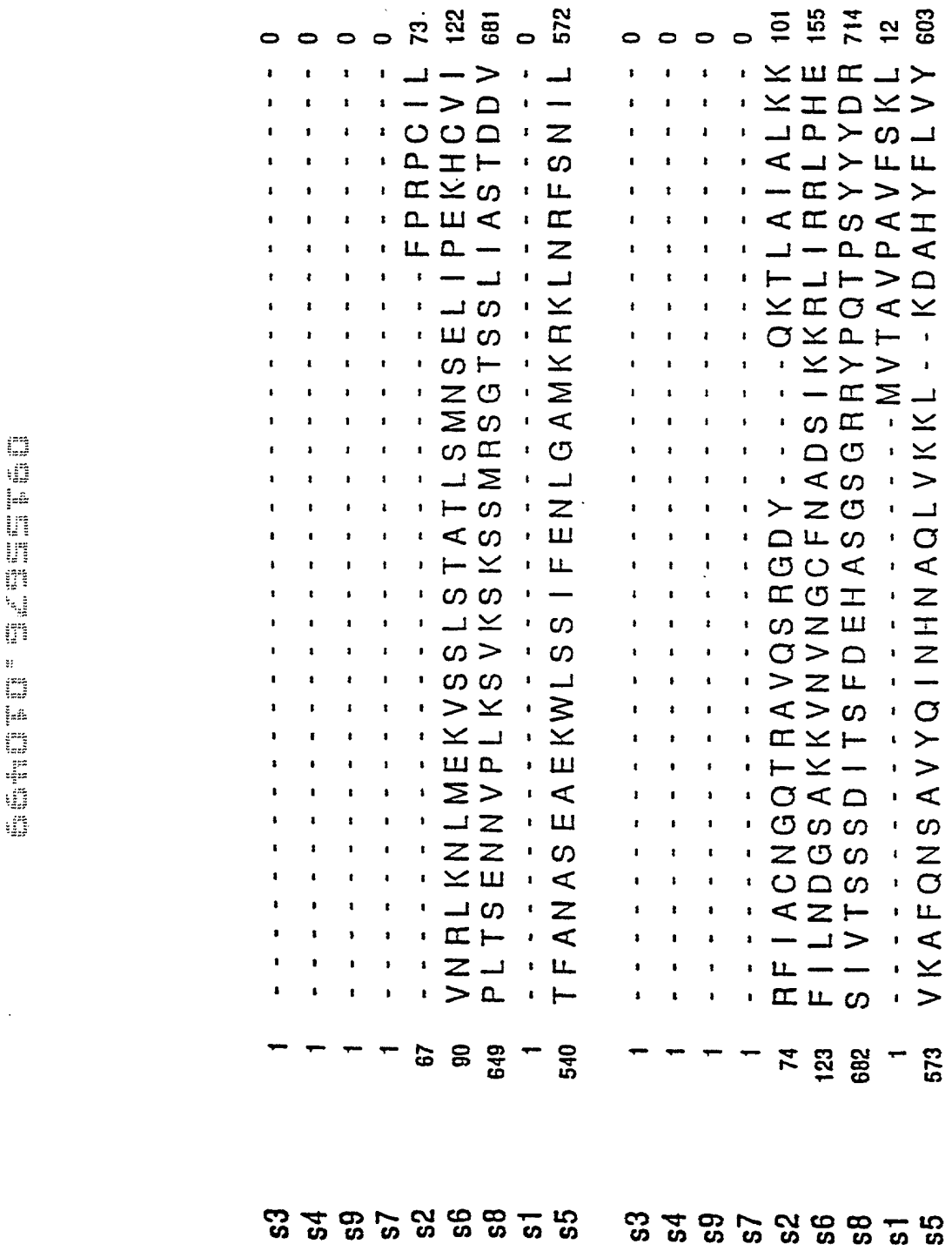
s3 1 ---------- 0
s4 1 ---------- 0
s9 1 ---------- 0
s7 1 ---------- 0
s2 67 ----------FPRPCIIL 73.
s6 90 VNRLKNLMEKVSSLSTATLSMNSELIPEKHCVI 122
s8 649 PLTSENNVPLKSVKSKSSMRSGTSSLIASTDDV 681
s1 1 ---------- 0
s5 540 TFANASEAEKWLSSIFENLGAMKRKLNRFSNIIL 572
s3 1 ---------- 0
s4 1 ---------- 0
s9 1 ---------- 0
s7 1 ---------- 0
s2 74 RFIACNGQTRAVQSRGDY----QKTLAIALKK 101
s6 123 FILNDGSAKKVNVNGCFNADSIKKRLIRRLPHE 155
s8 682 SIVTSSSDITSFDEHASGSGRRYPQTPSYYYDR 714
s1 1 ----------MVTAVPAVFSKL 12
s5 573 VKAFQNSAVYQINHNAQLVKKL--KDAHYFLVY 603

FIG. 7K

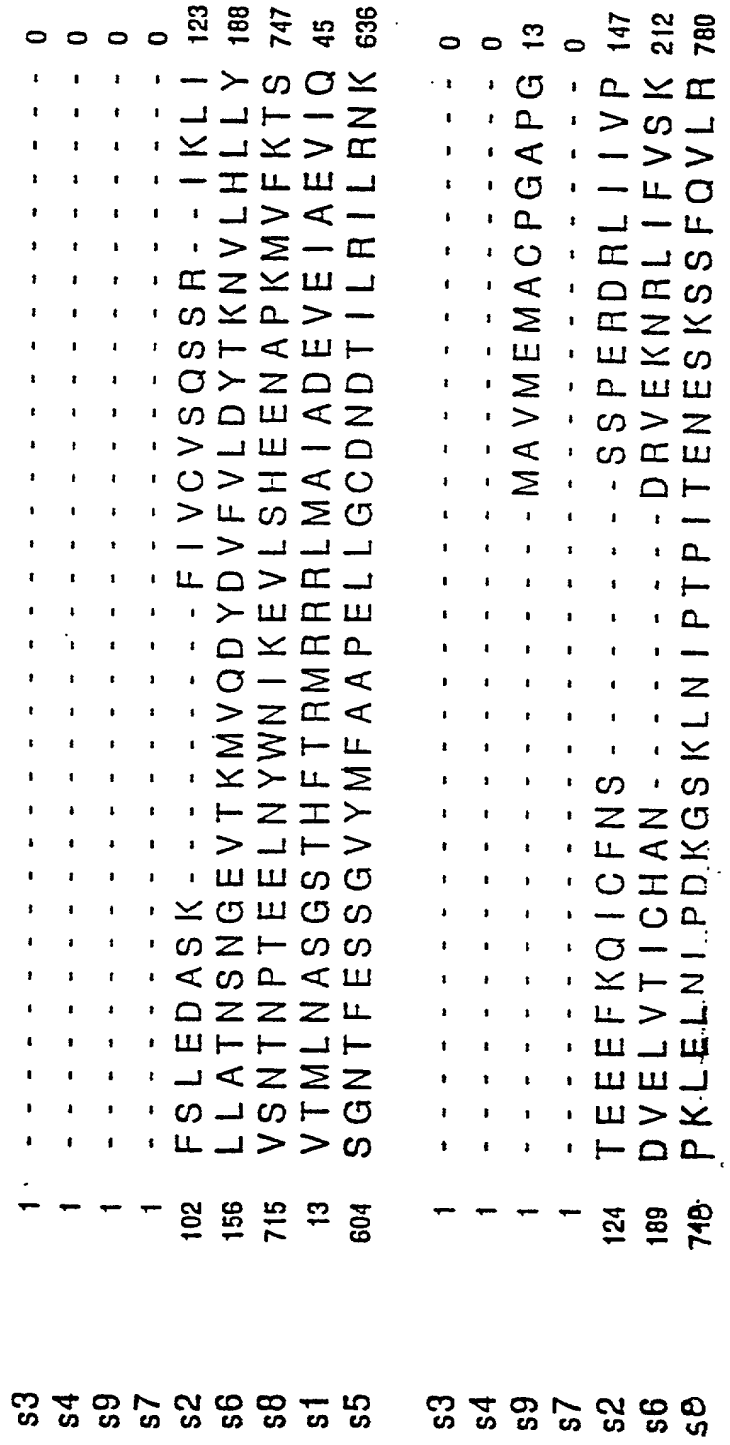
s3 1 ---------- 0
s4 1 ---------- 0
s9 1 ---------- 0
s7 1 ---------- 0
s2 102 FSLEDASK----------FIVCVSQSSR--IKLI 123
s6 156 LLATNSNGEVTKMVQDYDVFVLDYTKNVLHLLLY 188
s8 715 VSNTNPTEELNYWNIKEVLSHEENAPKMVFKTS 747
s1 13 VTMLNASGSTHFTRMRRRLMAIADEVEIAEVIQ 45
s5 604 SGNTFESSGVYMFAAPELLGCDNDTILRILRNK 636
s3 1 ---------- 0
s4 1 ---------- 0
s9 1 ----------MAVMEMACPGAPG 13
s7 1 ---------- 0
s2 124 TEEEFKQICFNS----------SSPERDRLIIVP 147
s6 189 DVELVTICHAN----------DRVEKNRLIFVSK 212
s8 748 PKLELNIPDKGSKLNIPTPITENESKSSFQVLR 780

FIG. 7L

```
s3    1  ---------------------------------  0
s4    1  ---------------------------------  0
s9   14  SAVGQQKELPKPKEKTPPLGKKQSSVYKLEAVE 46
s7    1  ---------------------------------  0
s2  148  KEKPCPSFEDLRRS-WEIELAQPAALSSQSSLS 179
s6  213  DQT-PSDKAISTS-KKLYL---RTLSALSQVGP 240
s8  781  KDEGTEIDFNHRRE-SPYTKPELAPKREAPKPP 812
s1   79  REKTGKGLSATRLSASSEDISDRLAGVSVGLPS 111
s5  670  KGEDSKGIPYYRVVANSSSDLDRHAHQSKKKNF 702

s3    1  ---------------------------------  0
s4    1  ---------------------------------  0
s9   47  KSPVFCGKWEILNDVITKGTAKEGSEAGPAAIS 79
s7    1  ---------------------------------  0
s2  180  PKLSS--VLPTSTQKRSVRSNNAKPFESY---- 206
s6  241  SSSN--LLA---QNKGISHNNAEGKLRIDNTEK 268
s8  813  ANTSPQRTLSTSKQNKPIRLVRASTKISRSKRS 845
s1  112  STTTEQPKPAVQTKGRPHSQCLNSSPLSHAQLM 144
s5  703  STDPFDQHLD-EKNNEVFELEVALSSLGALVVL 734
```

FIG. 7M

```
s3    1  --------------------------------- 0
s4    1  --------------------------------- 0
s9   80  IIAQAECENSQEFSPTFSERIFIAGSKQYSQSE 112
s7    1  --------------------------------- 0
s2  207  -------------------QRPPSELINSRISD 220
s6  269  DRIRQIFN----------QRPPSEFISTNLAG- 290
s8  846  KPLPPQLLSSPIEASSSSPDSLTSSYTPASTHV 878
s1  145  FPAPSAPCSSAPSVPDISKHRPQA-FVPCKIPS 176
s5  735  YPGEPVVWDGPVYKLPGNNLFASNEMDLGKIGN 767

s3    1  --------------------------------- 0
s4    1  --------------------------------- 0
s9  113  SLDQIPNNVAHATEGKMARVCWKGKRRSKARKK 145
s7    1  --------------------------------- 0
s2  221  ------FFPD------HQPKLLEKTISNSLRRN 241
s6  291  -----YFPH------TDMKRLQKTMRESFRHSA 312
s8  879  ------LIPQ-PYKGANDVMRRLKTDQDSTSTS 904
```

FIG. 7N

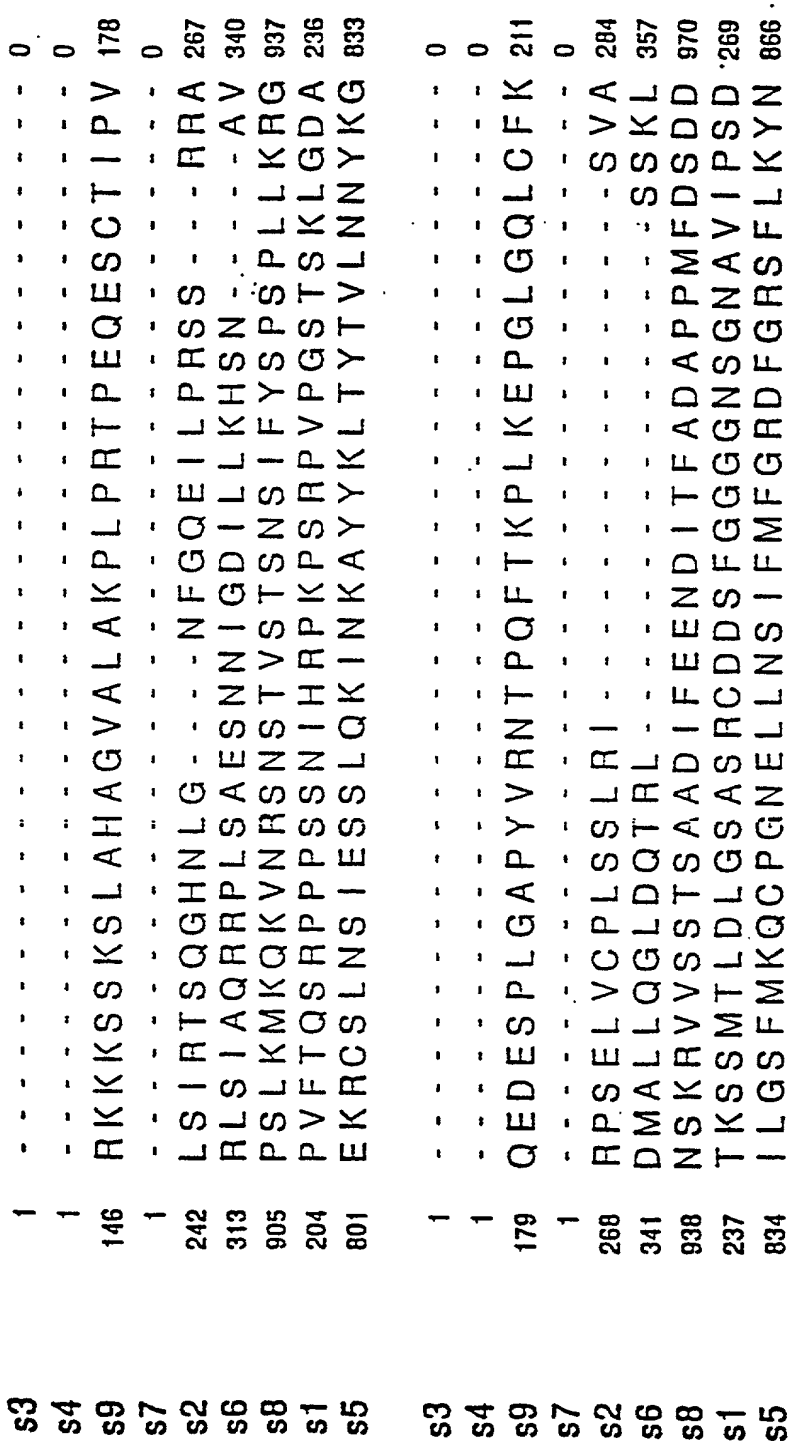
s3 1 ---------- 0
s4 1 ---------- 0
s9 146 RKKKSSKSLAHAGVALAKPLPRTPEQESCTIPV 178
s7 1 ---------- 0
s2 242 LSIRTSQGHNLG----NFGQEILPRSS----RRA 267
s6 313 RLSIAQRRPLSAESNNIGDILLKHSN-------AV 340
s8 905 PSLKMKQKVNRSNSTVSTSNSIFYSPSPLLKRG 937
s1 204 PVFTQSRPPSSNIHRPKPSRPVPGSTSKLGDA 236
s5 801 EKRCSLNSIESSLQKINKAYYKLTYTVLNNYKG 833
s3 1 ---------- 0
s4 1 ---------- 0
s9 179 QEDESPLGAPYVRNTPQFTKPLKEPGLGQLCFK 211
s7 1 ---------- 0
s2 268 RPSELVCPLSSLRI----------SVA 284
s6 341 DMALLQGLDQTRL----------SSKL 357
s8 938 NSKRVVSSTSAADIFEENDITFADAPPMFDSDD 970
s1 237 TKSSMTLDLGSASRCDDSFGGGGNSGNAVIPSD 269
s5 834 ILGSFMKQCPGNELLNSIFMFGRDFGRSFLKYN 866

FIG. 70

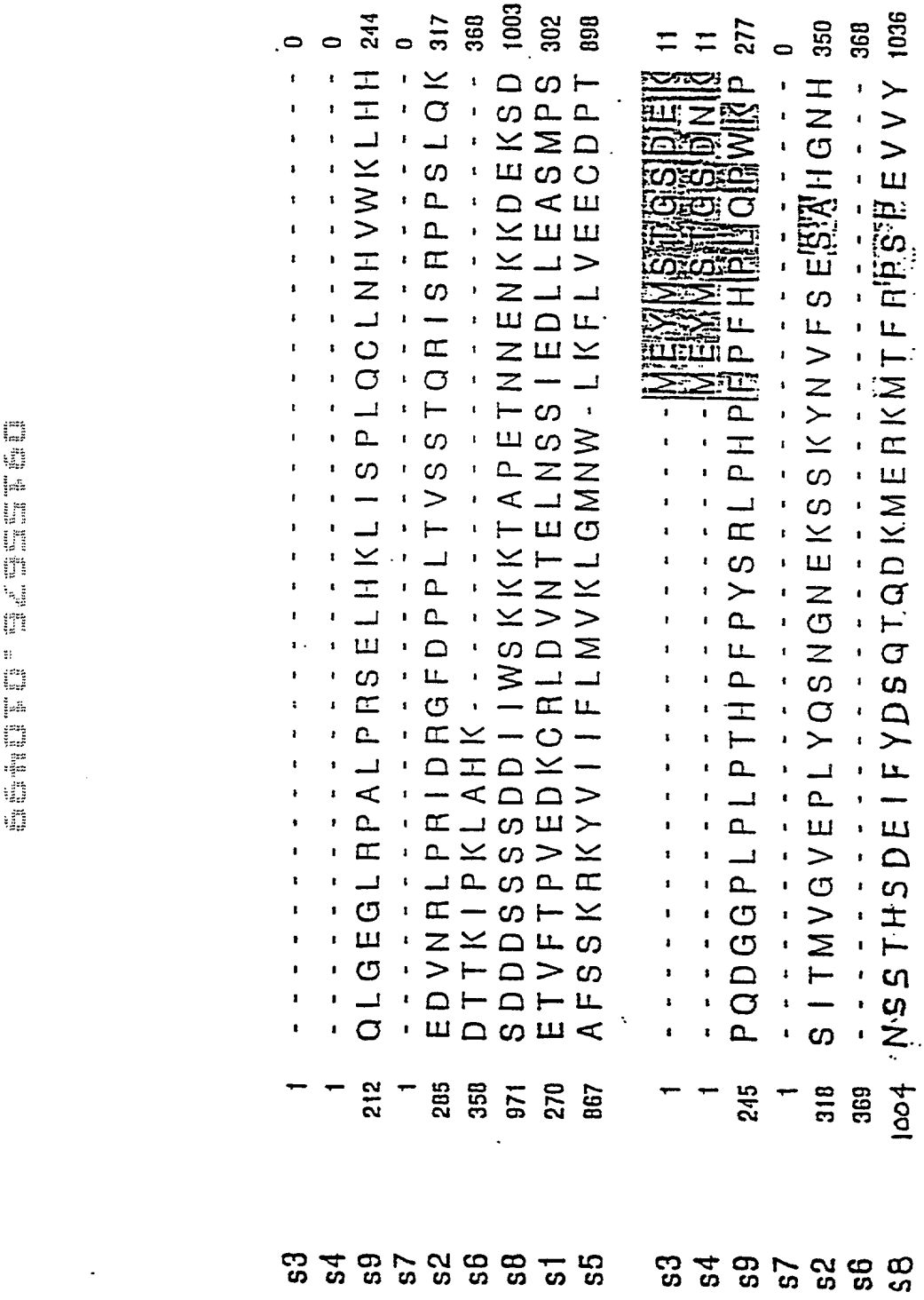
s3 1 ---------- 0
s4 1 ---------- 0
s9 212 QLGEGLRPALPRSELHKLISPLQCLNHVWKLHH 244
s7 1 ---------- 0
s2 285 EDVNRLPRIDRGFDPPLTVSSTQRISRPPSLQK 317
s6 358 DTTKIPKLAHK---------- 368
s8 971 SDDDSSSSDDIIWSKKKTAPETNNENKKDEKSD 1003
s1 270 ETVFTPVEDKCRLDVNTELNSSIEDLLEASMPS 302
s5 867 AFSSKRKYVIIFLMVKLGMNW-LKFLVEECDPT 898
s3 1 ---------- 11
s4 1 ---------- 11
s9 245 PQDGGPLPLPTHPFPYSRLPHPEPFHRLQPWKP 277
s7 1 ---------- 0
s2 318 SITMVGVEPLYQSNGNEKSSIKYNVFSESAHGNH 350
s6 369 ---------- 368
s8 1004 NSSTHSDEIFYDSQTQDKMERKMTFRRSPEVVY 1036

FIG. 7P

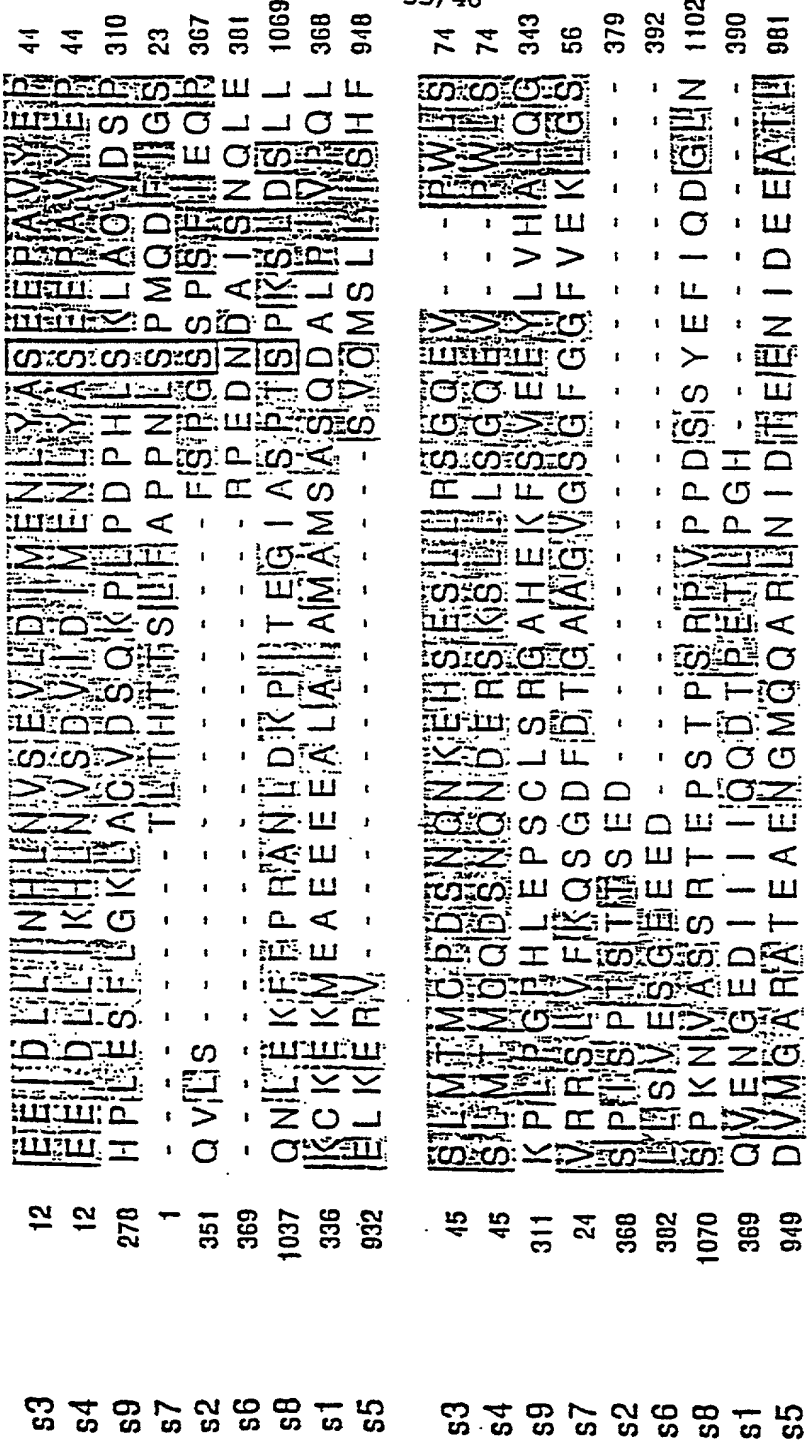

FIG. 7Q

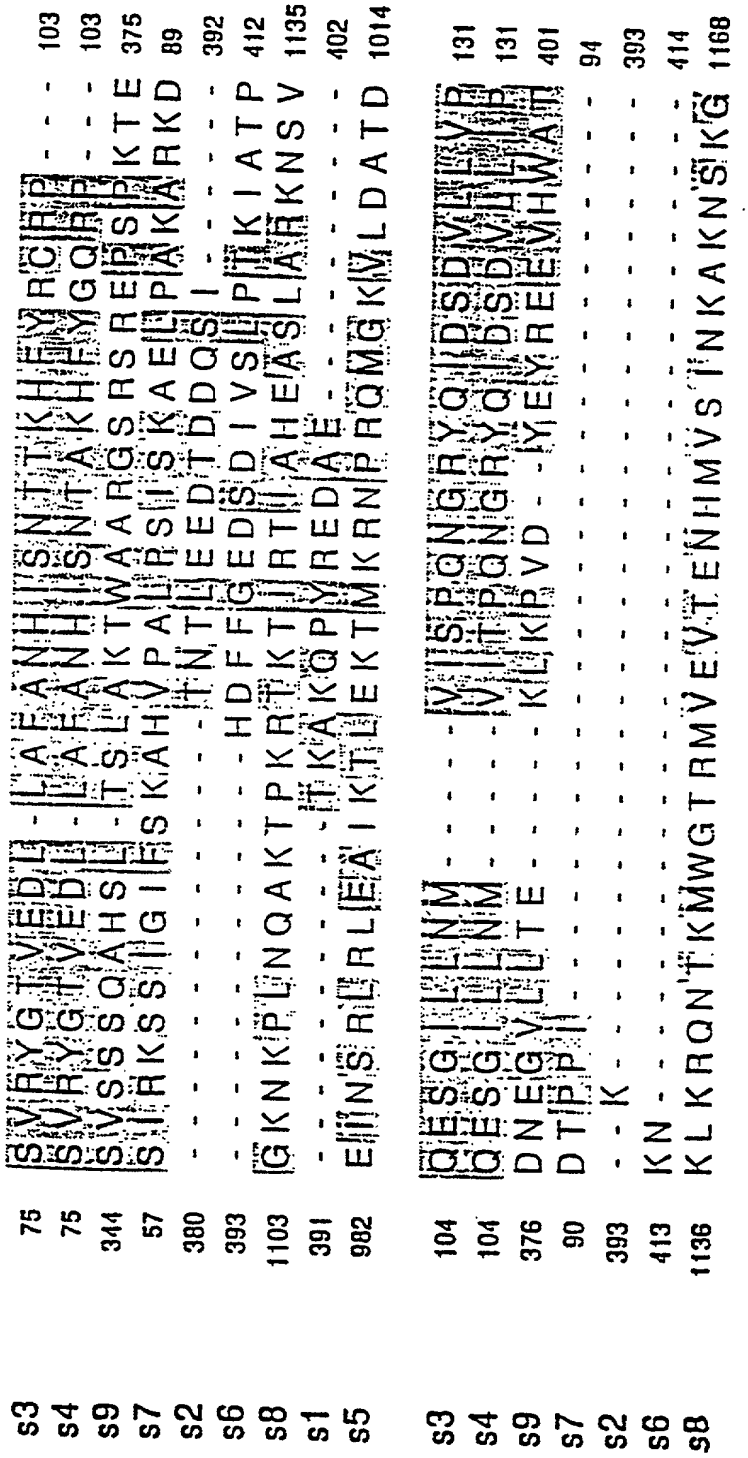
s3 75 S V R Y G T V E D L - L A E A N H I S N T T K H E Y R C R P - - - 103
s4 75 S V R Y G T V E D L - L A E A N H I S N T A K H F Y G Q R P - - - 103
s9 344 S V S S S Q A H S L - T S L A K T W A A R G S R S E P S P K T E 375
s7 57 S I R K S S I G I F S K A H V P A L P S I S K A E L P A K A R K D 89
s2 380 - - - - - - - - - - - - - - - - T N T L E E D T D D Q S I - - - - - - - 392
s6 393 - - - - - - - - - - - - - - - - H D F F G E D S D I V S L P I I K I A T P 412
s8 1103 G K N K P L N Q A K T P K R T T K T I R T I I A H E A S L A R K N S V 1135
s1 391 - - - - - - - - - - - - - - - - T K A K Q P Y R E D A E - - - - - - - - - 402
s5 982 E I I N S R L R L E A I K T L E K T M K R N P R Q M G K V L D A T D 1014
s3 104 Q E S G I L L N M - - - - - V I S P Q N G R Y Q I D S D V L L V R 131
s4 104 Q E S G I L L N M - - - - - V I T P Q N G R Y Q I D S D V I L I R 131
s9 376 D N E G V L L T E - - - - - K L K P V D - - Y E Y R E E V H W A T 401
s7 90 D T P P I - 94
s2 393 - - K - 393
s6 413 K N - 414
s8 1136 K L K R Q N T K M W G T R M V E V T E N H M V S I N K A K N S K G 1168

FIG. 7R

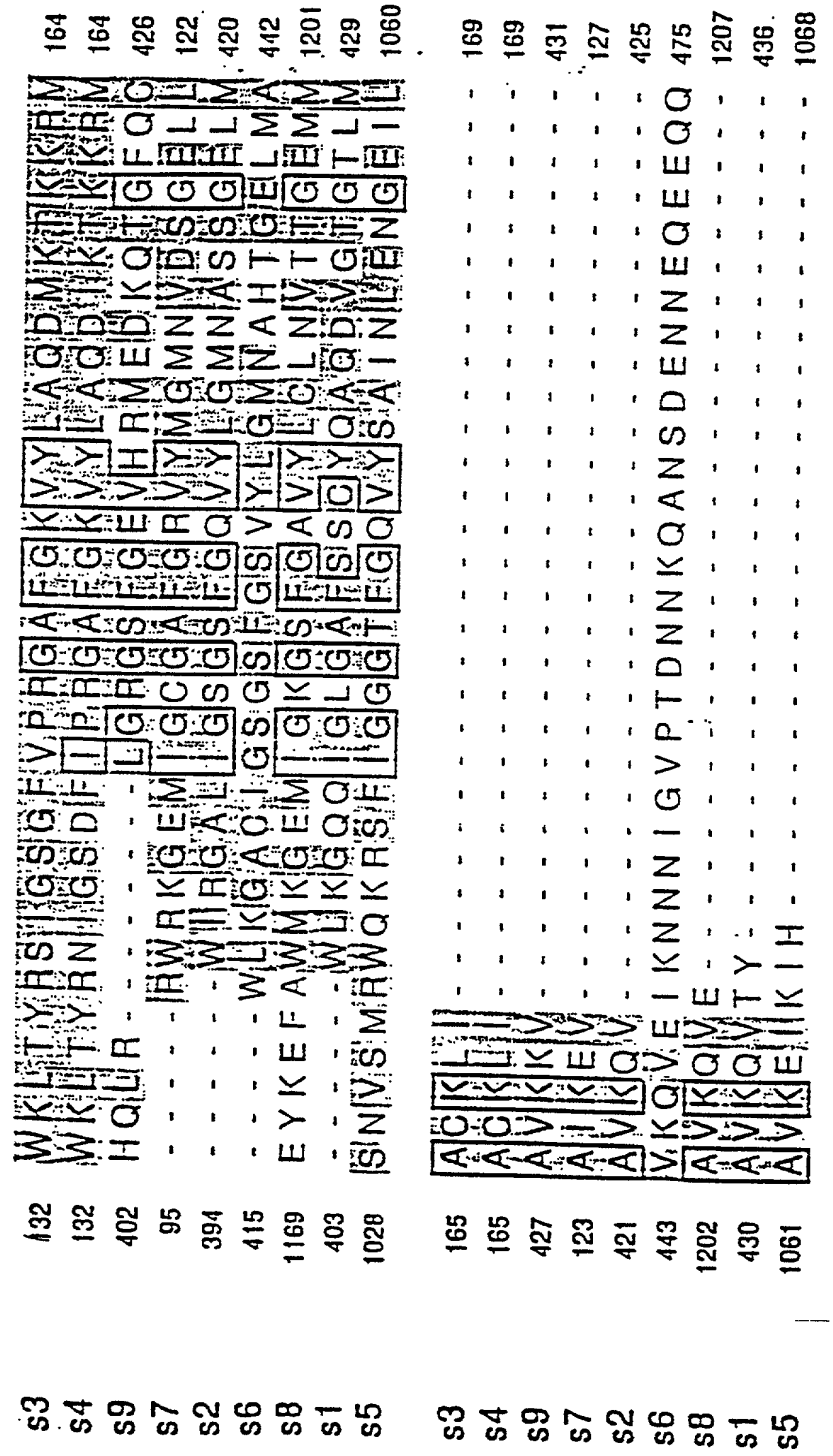

FIG. 7S

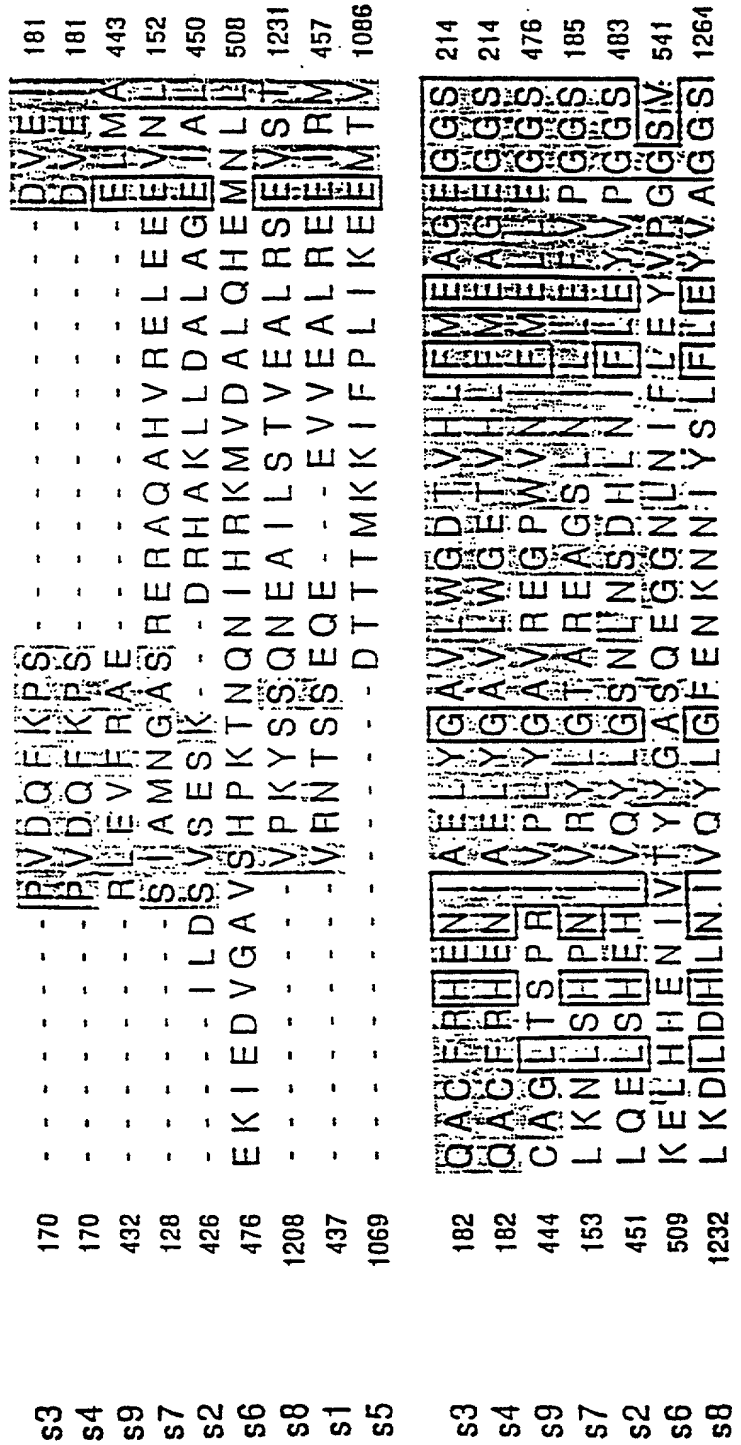

FIG. 7T

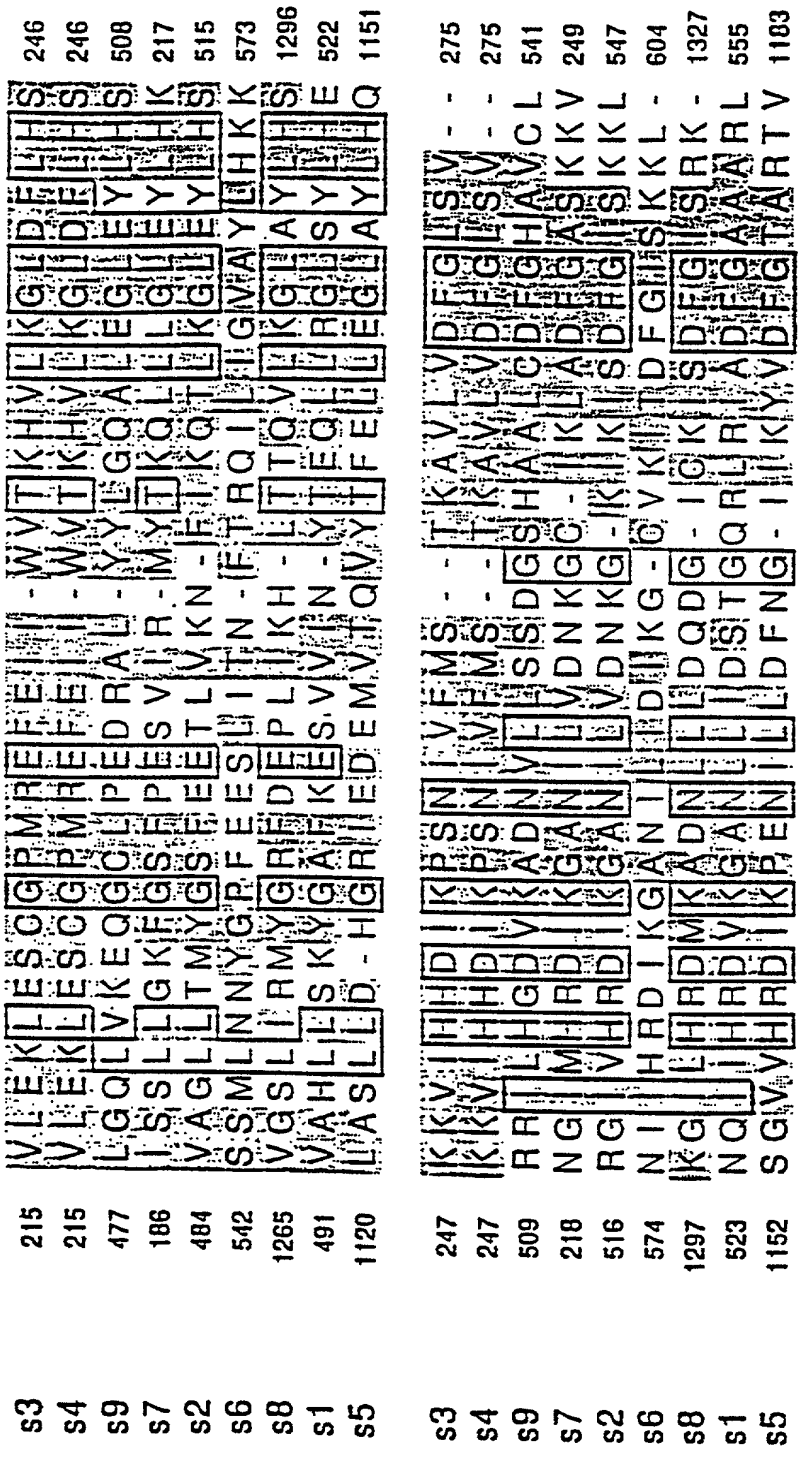

FIG. 7U

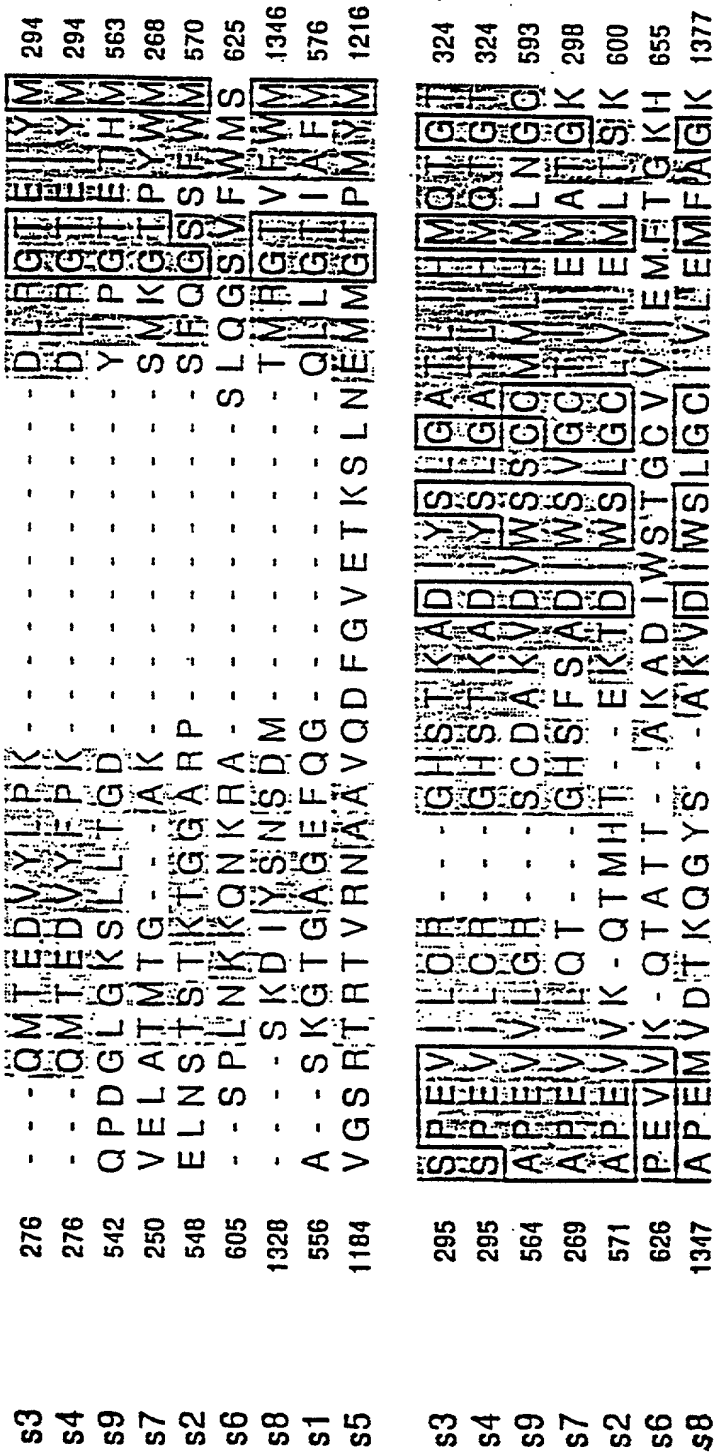

FIG. 7V

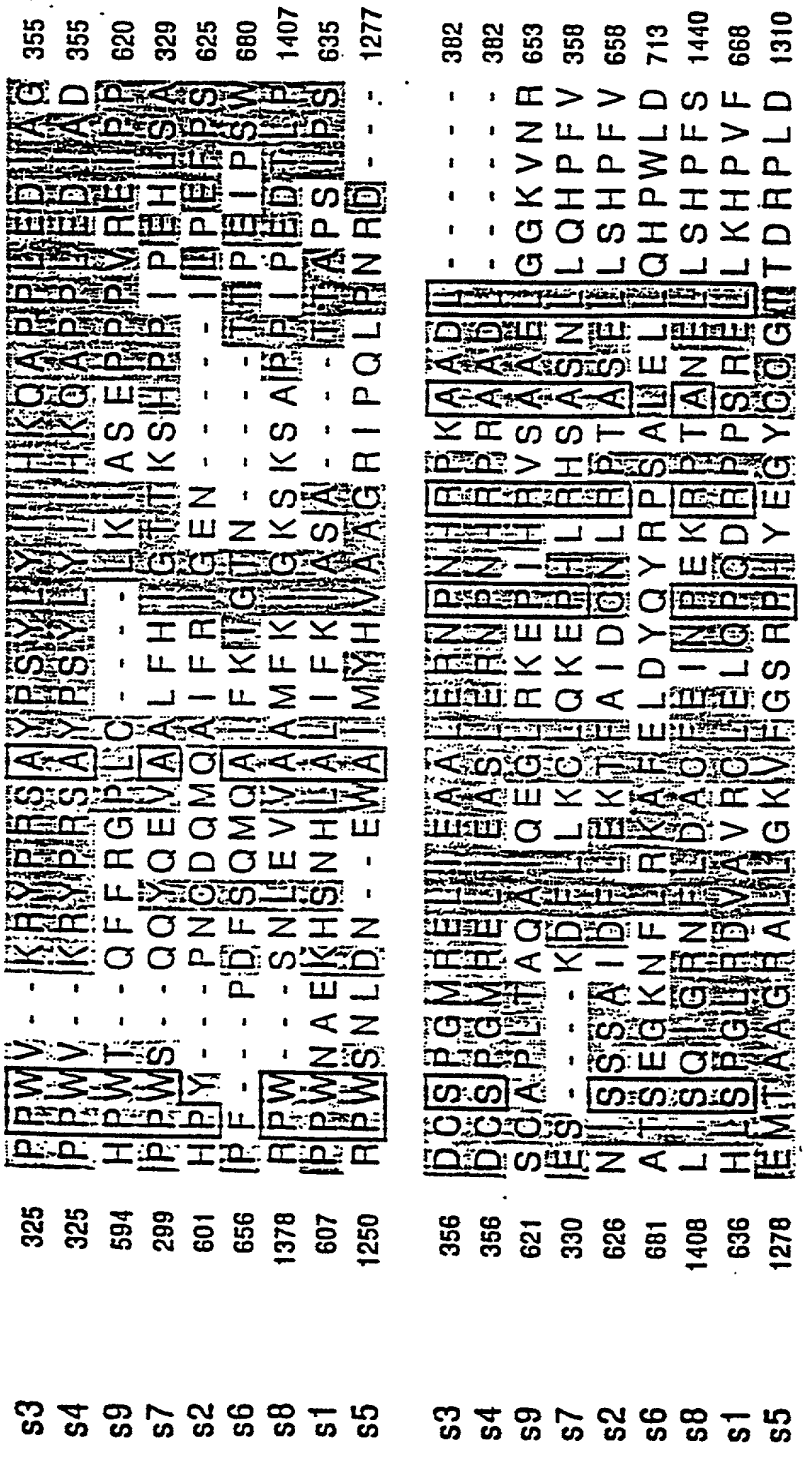

FIG. 7W

```
s3    383  -------LK---HEALNPPREDQPRQQSLDSAL  405
s4    383  -------LK---HEALNPPREDQPRQQSLDSAL  405
s9    654  ALQQVGGLKSPWRGEYKEPRHPPPNQANYHQTL  686
s7    359  TAEHQEARPFLRSSFMGNPENMAAQRMDVRTSI  391
s2    659  S--------------------------------  659
s6    714  AHII-----------------------------  717
s8   1441  EVNETFNFKSTRLAKFIKSNDKLNSSKLRITSQ  1473
s1    669  RTTW-----------------------------  672
s5   1311  DTNP-----------------------------  1314

s3    406  FDRKRLLSRKE---------------------L  417
s4    406  LERKRLLSRKE---------------------L  417
s9    687  HAQPRELSPRAPGPRPAEETTGRAPKLQPPLPP  719
s7    392  IPDMRASCNGLKDVCGVSAVRCSTVYPENSLGK  424
s2      0  ---------------------------------  659
s6      0  ---------------------------------  717
s8   1474  ENKTE----------------------------  1478
```

FIG. 7X

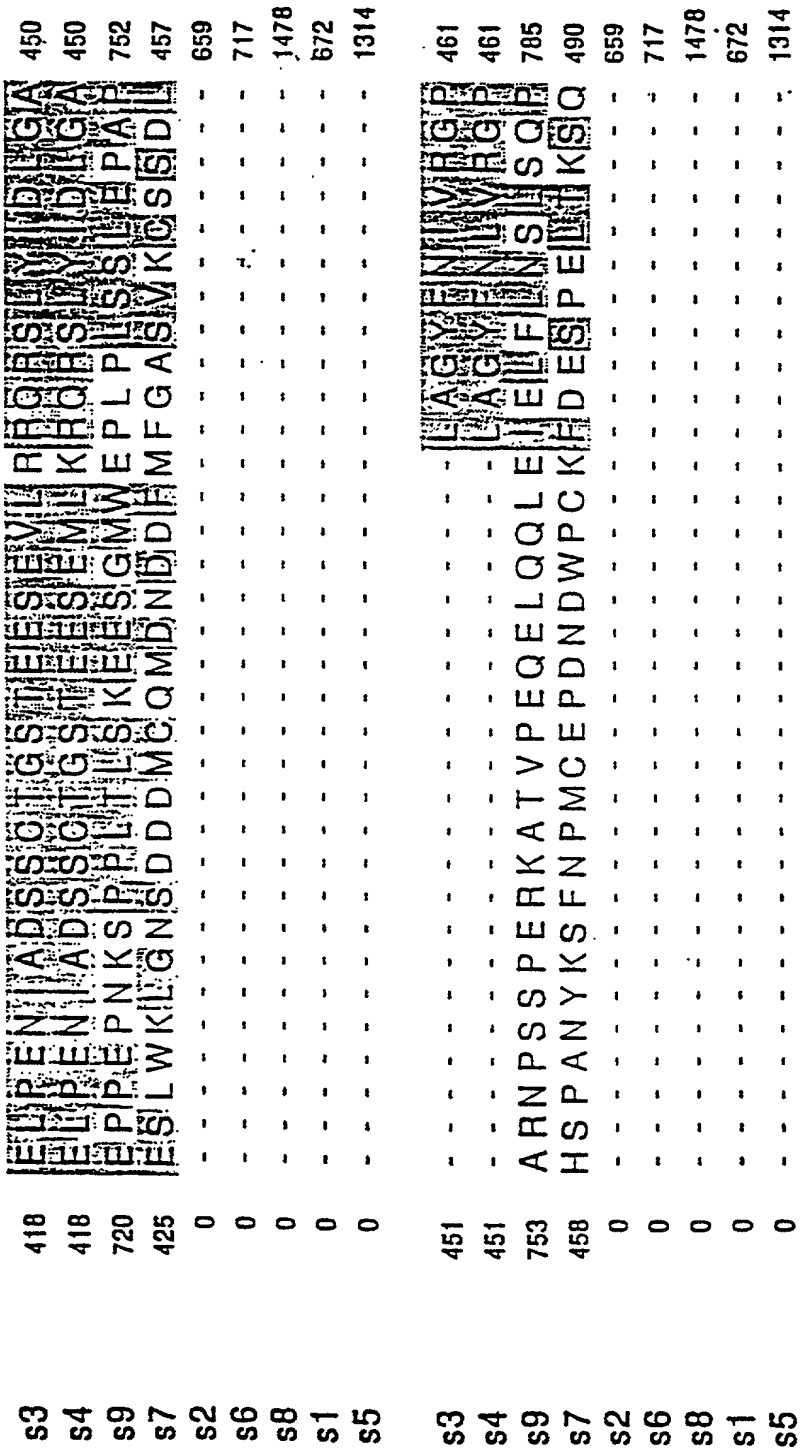

FIG. 7Y

```
s3   462  PTLEYG---------------------------  467
s4   462  PTLEYG---------------------------  467
s9   786  FSLEEQEQILSCLSIDSLSLSDDSEKNPSKASQ  818
s7   491  ANLHYDQATIKPTNNPIMSYKEDLAFTFPSGQS  523
s2     0  ---------------------------------  659
s6     0  ---------------------------------  717
s8     0  ---------------------------------  1478
s1     0  ---------------------------------  672
s5     0  ---------------------------------  1314

s3     0  ---------------------------------  467
s4     0  ---------------------------------  467
s9   819  SSRDTLSSGVHSWSSQAEARSSSWNMVLARGRP  851
s7   524  AAEDDDELTESKIRAFLDEKAMDLKKLQTPLYE  556
s2     0  ---------------------------------  659
s6     0  ---------------------------------  717
s8     -  ---------------------------------  1478
```

FIG. 7Z

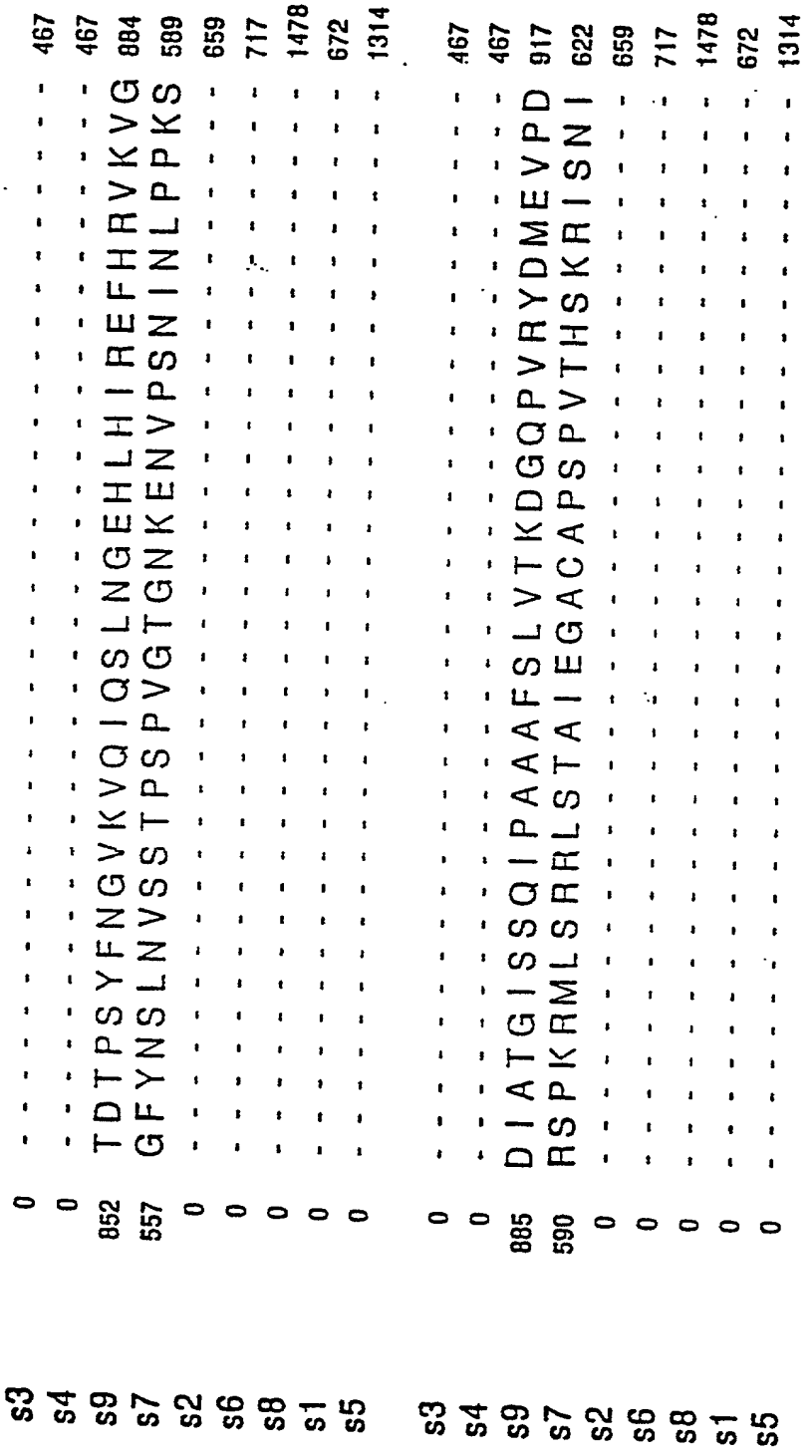
s3 0 ---------- 467
s4 0 ---------- 467
s9 852 TDTPSYFNGVKVQIQSLNGEHLHIREFHRVKVG 884
s7 557 GFYNSLNVSSTPSPVGTGNKENVPSNINLPPKS 589
s2 0 ---------- 659
s6 0 ---------- 717
s8 0 ---------- 1478
s1 0 ---------- 672
s5 0 ---------- 1314
s3 0 ---------- 467
s4 0 ---------- 467
s9 885 DIATGISSQIPAAAFSLVTKDGQPVRYDMEVPD 917
s7 590 RSPKRMLSRRLSTAIEGACAPSPVTHSKRISNI 622
s2 0 ---------- 659
s6 0 ---------- 717
s8 0 ---------- 1478
s1 0 ---------- 672
s5 0 ---------- 1314

FIG. 7AA

```
s3    0  ---------------------------------  467
s4    0  ---------------------------------  467
s9  918  SGIDLQCTLAPDGSFAWSWRVKHGQLENRP---  947
s7  623  GGLNGEAIQEAQLPRHNEWKDLLGSQREAVNSS  655
s2    0  ---------------------------------  659
s6    0  ---------------------------------  717
s8    0  ---------------------------------  1478
s1    0  ---------------------------------  672
s5    0  ---------------------------------  1314

s3    0  ---------------------------------  467
s4    0  ---------------------------------  467
s9    0  ---------------------------------  947
s7  656  FSERQRRWKEELDEELQRKREIMRQAVNLSPPK  688
s2    0  ---------------------------------  659
s6    0  ---------------------------------  717
s8    0  ---------------------------------  1478
```

FIG. 7BB

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Yeda Research and Development Co. Ltd.
(B) STREET: Weizmann Institute of Science
(C) CITY: P.O.B. 95
(D) STATE: Rehovot
(E) COUNTRY: Israel
(F) POSTAL CODE (ZIP): 76100
(G) TELEPHONE: +972-8-9344093
(H) TELEFAX: +972-8-9470739

(A) NAME: David Wallach
(B) STREET: 24 Borochov Street
(C) CITY: Rehovot
(E) COUNTRY: Israel
(F) POSTAL CODE (ZIP): 76406

(A) NAME: Nikolai Malinin
(B) STREET: Beit Clore, Weizmann Institute of Science
(C) CITY: Rehovot
(E) COUNTRY: Israel
(F) POSTAL CODE (ZIP): 76100

(A) NAME: Mark Boldin
(B) STREET: Beit Clore, Weizmann Institute of Science
(C) CITY: Rehovot
(E) COUNTRY: Israel
(F) POSTAL CODE (ZIP): 76100

(A) NAME: Andrei Kovalenko
(B) STREET: Beit Clore, Weizmann Institute of Science
(C) CITY: Rehovot
(E) COUNTRY: Israel
(F) POSTAL CODE (ZIP): 76100

(A) NAME: Igor Mett
(B) STREET: 60 Levin Epstein Street
(C) CITY: Rehovot
(E) COUNTRY: Israel
(F) POSTAL CODE (ZIP): 76462

(ii) TITLE OF INVENTION: Modulators of TNF Receptor Associated Factor (TRAF), their Preparation and Use

(iii) NUMBER OF SEQUENCES: 11

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(v) CURRENT APPLICATION DATA:
APPLICATION NUMBER: PCT/IL97/00117
(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: IL 117800
(B) FILING DATE: 02-APR-1996

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: IL 119133
(B) FILING DATE: 26-AUG-1996

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1906 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

CATTGGGTCA CGCGGTGGCG GCGCTCTAGA ATAGTGGATC CCCCGGGCTG CAGGAATTCG 60

ATTCGAGGCC ACGAAGGCCG GCGGCGCGGC GCANGCACCG GCCCGGGGAN AGGCNCCATG 120

AGCGGATCNC NGAACNATGA CAAAAGACAA TTTCTGCTGG AGCGACTGCT GGATGCAGTG 180

AAACAGTGCC AGATCCGCTT TNGAGGGAGA AAGGAGATTG CCTCGGATTC CGACAGCAGG 240

GTCACCTGTC TGTGTGCCCA GTTTGAAGCC GTCCTGCAGC ATGGCTTGAA GAGGAGTCGA 300

GGATTGGCAC TCACAGCGGC AGCGATCAAG CAGGCAGCGG GCTTTGCCAG CAAAACCGAA 360

ACAGAGCCCG TGTTCTGGTA CTACGTGAAG GAGGTCCTCA ACAAGCACGA GCTGCAGCGC 420

TTCTACTCCC TGCGCCACAT CGCCTCAGAC GTGGGCCGGG GTCGCGCCTG GCTGCGCTGT 480

GCCCTCAACG AACACTCCCT GGAGCGCTAC CTGCACATGC TCCTGGCCGA CCGCTGCAGG 540

CTGAGCACTT TTTATGAAGA CTGGTCTTTT GTGATGGATG AAGAAAGGTC CAGTATGCTT 600

CCTACCATGG CAGCAGGTCT GAACTCCATA CTCTTTGCGA TTAACATCGA CAACAAGGAT 660

TTGAACGGGC AGAGTAAGTT TGCTCCCACC GTTTCAGACC TCTTAAAGGA GTCAACGCAG 720

AACGTGACCT CCTTGCTGAA GGAGTCCACG CAAGGAGTGA GCAGCCTGTT CAGGGAGATC 780

ACAGCCTCCT CTGCCGTCTC CATCCTCATC AAACCTGAAC AGGAGACCGA CCCTTGCCTG 840

TCGTGTCCAG GAATGTCAGT GCTGATGCCA AATGCAAAAA GGAGCGGAAG AAGAAAAAGA 900

AAGTGACCAA CATAATCTCA TTTGATGATG AGGAAGATGA GCAGAACTCT GGGGACGTGT 960

TTAAAAAGAC ACCTGGGGCA GGGGAGAGCT CAGAGGACAA CTCCGACCGC TCCTCTGTCA 1020

ATATCATGTC CGCCTTTGAA AGCCCCTTCG GGCCTAACTC CAATGGAATC AGAGCAGCAA 1080

CTCATGGAAA ATTGATTCCC TGTCTTTGAA CGGGGAGTTT GGGTACCAGA AGCTTGATGT 1140

GAAAAGCATC GATGATGAAG ATGTGGATGA AAACGAAGAT GACGTGTATG GAAACTCATC 1200

AGGAAGGAAG CACAGGGGCC ACTCGGAGTC GCCCGAGAAG CCACTGGAAG GGAACACCTG 1260

CCTCTCCCAG ATGCACAGCT GGGCTCCGCT GAAGGTGCTG CACAATGACT CCGACATCCT 1320

CTTCCCTGTC AGTGGCGTGG GCTCCTACAG CCCAGCAGAT GCCCCCCTCG GAAGCCTGGA 1380

GAACGGGACA GGACCAGAGG ACCACGTTCT CCCGGATCCT GGACTTCGGT ACAGTGTGGA 1440

AGCCAGCTCT CCAGGCCACG GAAGTCCTCT GAGCAGCCTG TTACTTCTGC CTCAGTGCCA 1500

GAGTCCATGA CAATTAGTGA ACTGCGCCAG GCCACTGTGG CCATGATGAA CAGGAAGGAT 1560

GAGCTGGAGG AGGAGAACAG ATCACTGCGA AACCTGCTCG ACGGTGAGAT GGAGCACTCA 1620

GCCGCGCTCC GGCAAGAGGT GGACACCTTG AAAAGGAAGG TGGCTGAACA GGAGGAGCGG 1680

CAGGGCATGA AGGTCCAGGC GCTGGCCAGC TATCTTTGCT ATTTTGTGAG GAGATTCTAA 1740

CCCCACGTGA GAACCATGTG GTGGAGAAAT GGAGGGAGAG AGAAATCCAA CAGTTCCTGA 1800

TAGTCTCATT TGAGCTCCTG GATCCAGTCT TTCCTGAAGC TGTGTTTCCT CTGGACTTTT 1860

CATGTATGTG AGCCAATAAA TTGCTTTCAT TCCTTGAAAA AAAAAA 1906

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 604 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Xaa Thr Gly Pro Gly Xaa Gly Xaa Met Ser Gly Ser Xaa Asn Xaa Asp
1 5 10 15

Lys Arg Gln Phe Leu Leu Glu Arg Leu Leu Asp Ala Val Lys Gln Cys
20 25 30

Gln Ile Arg Phe Xaa Gly Arg Lys Glu Ile Ala Ser Asp Ser Asp Ser
35 40 45

Arg Val Thr Cys Leu Cys Ala Gln Phe Glu Ala Val Leu Gln His Gly
50 55 60

Leu Lys Arg Ser Arg Gly Leu Ala Leu Thr Ala Ala Ala Ile Lys Gln
65 70 75 80

Ala Ala Gly Phe Ala Ser Lys Thr Glu Thr Glu Pro Val Phe Trp Tyr
85 90 95

Tyr Val Lys Glu Val Leu Asn Lys His Glu Leu Gln Arg Phe Tyr Ser
100 105 110

Leu Arg His Ile Ala Ser Asp Val Gly Arg Gly Arg Ala Trp Leu Arg

```
        115                 120                 125

Cys Ala Leu Asn Glu His Ser Leu Glu Arg Tyr Leu His Met Leu Leu
    130                 135                 140

Ala Asp Arg Cys Arg Leu Ser Thr Phe Tyr Glu Asp Trp Ser Phe Val
145                 150                 155                 160

Met Asp Glu Glu Arg Ser Ser Met Leu Pro Thr Met Ala Ala Gly Leu
                165                 170                 175

Asn Ser Ile Leu Phe Ala Ile Asn Ile Asp Asn Lys Asp Leu Asn Gly
            180                 185                 190

Gln Ser Lys Phe Ala Pro Thr Val Ser Asp Leu Leu Lys Glu Ser Thr
        195                 200                 205

Gln Asn Val Thr Ser Leu Leu Lys Glu Ser Thr Gln Gly Val Ser Ser
    210                 215                 220

Leu Phe Arg Glu Ile Thr Ala Ser Ser Ala Val Ser Ile Leu Ile Lys
225                 230                 235                 240

Pro Glu Gln Glu Thr Asp Pro Cys Leu Ser Cys Pro Gly Met Ser Val
                245                 250                 255

Leu Met Pro Asn Ala Lys Arg Ser Gly Arg Arg Lys Arg Lys Xaa Pro
            260                 265                 270

Thr Xaa Ser His Leu Met Met Arg Lys Met Ser Arg Thr Leu Gly Thr
        275                 280                 285

Cys Leu Lys Arg His Leu Gly Gln Gly Arg Ala Gln Arg Thr Thr Pro
    290                 295                 300

Thr Ala Pro Leu Ser Ile Ser Cys Pro Pro Leu Lys Ala Pro Ser Gly
305                 310                 315                 320

Leu Thr Pro Met Glu Ser Glu Gln Gln Leu Met Glu Asn Xaa Phe Pro
                325                 330                 335

Val Phe Glu Arg Gly Val Trp Val Pro Glu Ala Xaa Cys Glu Lys His
            340                 345                 350

Arg Xaa Xaa Arg Cys Gly Xaa Lys Arg Arg Xaa Arg Val Trp Lys Leu
        355                 360                 365

Ile Arg Lys Glu Ala Gln Gly Pro Leu Gly Val Ala Arg Glu Ala Thr
    370                 375                 380

Gly Arg Glu His Leu Pro Leu Pro Asp Ala Gln Leu Gly Ser Ala Glu
385                 390                 395                 400

Gly Ala Ala Gln Xaa Leu Arg His Pro Leu Pro Cys Gln Trp Arg Gly
                405                 410                 415

Leu Leu Gln Pro Ser Arg Cys Pro Pro Arg Lys Pro Gly Glu Arg Asp
            420                 425                 430

Arg Thr Arg Gly Pro Arg Ser Pro Gly Ser Trp Thr Ser Val Gln Cys
        435                 440                 445
```

Gly Ser Gln Leu Ser Arg Pro Arg Lys Ser Ser Glu Gln Pro Val Thr
450 455 460

Ser Ala Ser Val Pro Glu Ser Met Thr Ile Ser Glu Leu Arg Gln Ala
465 470 475 480

Thr Val Ala Met Met Asn Arg Lys Asp Glu Leu Glu Glu Glu Asn Arg
485 490 495

Ser Leu Arg Asn Leu Leu Asp Gly Glu Met Glu His Ser Ala Ala Leu
500 505 510

Arg Gln Glu Val Asp Thr Leu Lys Arg Lys Val Ala Glu Gln Glu Glu
515 520 525

Arg Gln Gly Met Lys Val Gln Ala Leu Ala Ser Tyr Leu Cys Tyr Phe
530 535 540

Val Arg Arg Phe Xaa Pro His Val Arg Thr Met Trp Trp Arg Asn Gly
545 550 555 560

Gly Arg Glu Lys Ser Asn Ser Ser Xaa Xaa Ser His Leu Ser Ser Trp
565 570 575

Ile Gln Ser Phe Leu Lys Leu Cys Phe Leu Trp Thr Phe His Val Cys
580 585 590

Glu Pro Ile Asn Cys Phe His Ser Leu Lys Lys Lys
595 600

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2631 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CCCCTCTCAC AGCCCAGGCC ATCCAAGAGG GGCTGAGGAA AGAGCCCATC CACCGCGTGT 60

CTGCAGCGGA GCTGGGAGGG AAGGTGAACC GGGCACTACA GCAAGTGGGA GGTCTGAAGA 120

GCCCTTGGAG GGGAGAATAT AAAGAACCAA GACATCCACC GCCAAATCAA GCCAATTACC 180

ACCAGACCCT CCATGCCCAG CCGAGAGAGC TTTCGCCAAG GGCCCCAGGG CCCCGGCCAG 240

CTGAGGAGAC AACAGGCAGA GCCCCTAAGC TCCAGCCTCC TCTCCCACCA GAGCCCCCAG 300

AGCCAAACAA GTCTCCTCCC TTGACTTTGA GCAAGGAGGA GTCTGGGATG TGGGAACCCT 360

TACCTCTGTC CTCCCTGGAG CCAGCCCCTG CCAGAAACCC CAGCTCACCA GAGCGGAAAG 420

CAACCGTCCC GGAGCAGGAA CTGCAGCAGC TGGAAATAGA ATTATTCCTC AACAGCCTGT 480

CCCAGCCATT TTCTCTGGAG GAGCAGGAGC AAATTCTCTC GTGCCTCAGC ATCGACAGCC 540

TCTCCCTGTC GGATGACAGT GAGAAGAACC CATCAAAGGC CTCTCAAAGC TCGCGGGACA 600

CCCTGAGCTC AGGCGTACAC TCCTGGAGCA GCCAGGCCGA GGCTCGAAGC TCCAGCTGGA 660

ACATGGTGCT GGCCCGGGGG CGGCCCACCG ACACCCCAAG CTATTTCAAT GGTGTGAAAG 720

TCCAAATACA GTCTCTTAAT GGTGAACACC TGCACATCCG GGAGTTCCAC CGGGTCAAAG 780

TGGGAGACAT CGCCACTGGC ATCAGCAGCC AGATCCCAGC TGCAGCCTTC AGCTTGGTCA 840

CCAAAGACGG GCAGCCTGTT CGCTACGACA TGGAGGTGCC AGACTCGGGC ATCGACCTGC 900

AGTGCACACT GGCCCCTGAT GGCAGCTTCG CCTGGAGCTG GAGGGTCAAG CATGGCCAGC 960

TGGAGAACAG GCCCTAACCC TGCCCTCCAC CGCCGGCTCC ACACTGCCGG AAAGCAGCCT 1020

TCCTGCTCGG TGCACGATGC TGCCCTGAAA ACACAGGCTC AGCCGTTCCC AGGGGATYTG 1080

NCCAGCCCCC CGGCTCARCA GNTGGGAACC AGGGCCTCGN CAGCNAGCNA AGGTNGGGGG 1140

CAAGCNAGAA TGCCTCCCAG GATTTCACAN CCTGAGCCCN TGCCCCANCC CTGCTGAADA 1200

AAACAYTNCC GCCACGTGAA GAGACAGAAG GAGGATGGNC AGGAGTTNNA CCTYGGGGAA 1260

ACAAAACAGG GATCTTTNTT CTGCCCCTGC TCCAGTNCGA GTTGGCCTGN ACCCGCTTGG 1320

ANTCAGTGAC CATTTGTTGG CAGANCAGGG GAGAGCAGCT TCCAGCCTGG GTCAGAAGGG 1380

GTGGGCGAGC CCTTCGGCCC CTCACCCTNC CAGGCTGCTG TGNAGAGTGT CAAGTGTGTA 1440

AGGGNCCCAA ANCTCAGGNT TCAGTGCAGA ACCAGGTNCA GCAGGTATGC CCGCCCGNTA 1500

GGTTAANNGG GGGCCCTCTN AAACCCCTTG CCTNGGCCTN CACCTNGGCC AGCTCANCCC 1560

CTTTTGGGTG TAGGGGAAAA GAATGCCTGA CCCTGGGAAG GCTWCCCTGG TAGAATACAC 1620

CACACTTTTC AGGTTGTTGC AACACAGGTC CTGAGTTGAC CTCTGGTTCA GCCAAGGACC 1680

AAAGAAGGTG TGTAAGTGAA GTGGTTCTCA GTNCCCCAGA CATGTGCCCC TTTGCTGCTG 1740

GCTACCACTC TTCCCCAGAG CAGCAGGCCC CGAGCCCCTT CAGGCCCAGC ACTGCCCCAG 1800

ACTCGCTGGC ACTCAGTTCC CTCATCTGTA AAGGTGAAGG GTGATGCAGG ATATGCCTGA 1860

CAGGAACAGT CTGTGGATGG ACATGATCAG TGCTNAAGGN AAAGCAGCAG AGAGAGACGY 1920

TCCGGCGCCC CAGNCCCCAC TNATCAGTGT NCCAGCGTGC TNGGTTNCCC CAGNAGCACA 1980

GCTNCAGNCA TCANCACTGA CACTNCACCC TNGCCCTGCC CCTNGGCCAN GAGGGTACTG 2040

CCGNACGGCA CTTTGCACNT CTGATGNACC TCAAAGCACT TTCATGGCTN GCCCTCTNNG 2100

GCAGGGNCAG GGNCAGGGNC AGTGACANCT GTAGGNAGCA TANGCAANGC CAGGAGATGG 2160

GGTGNAAGGG ANCACAGTCT TGAGCTGTCC ANCATGCATG TGACTNCCTC AAACCTCTTN 2220

NCCAGNATTT CTCTAAGAAT AGCANCCCCC TTNCCCCATT GCCCCAGCTT AGCCTCTTCT 2280

CCCAGGGGAG CTANCTCAGG ACTCACGTAG CATTAAATCA GCTGTGNAAT CGTCAGGGGG 2340

TGTCTGCTAG CCTCAACCTC CTGGGGCAGG GGACGCCGAG ACTCCGTGGG AGAAGCTCAT 2400

TCCCACATCT TGCCAAGACA GCCTTTNGTC CAGCTGTCCA CATTGAGTCA GACTGCTCCC 2460

GGGGAGAGAG CCCCGGCCCC CAGCACATAA AGAACTGCAG CCTTGGTACT GCAGAGTCTG 2520

GGTTGTAGAG AACTCTTTGT AAGCAATAAA GTTTGGGGTG ATGACAAATG TTAAAAAAAG 2580

GCCTTCGTGG CCTCGAATCA AGCTTATCGA TACCGTCGAC CTCGAGGGGG G 2631

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1253 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

CATTGGAGTC ACGCGGTGGC GGCGCTCTAG AATAGTGGAT CCCCGGGCTG CANGGAATTC 60

GATTCGAGCC CACGAAGGCC CCTTCTTCTG TGGTCGCGGC ACGTTTACAG CCGCAAGCAC 120

CCAGCGGCAG CTGAAGGAGG CTTTTGAGAG GCTCCTGCCC CAGGTGGAGG CGGCCCGCAA 180

GGCCATCCGC GCCGCTCAGG TGGAGCGCTA TGTGCCCGAA CACGAGCGAT GCTGCTGGTG 240

CCTGTGCTGC GGCTGTGAGG TGCGGGAACA CCTGAGCCAT GGAAACCTGA CGGTGCTGTA 300

CGGGGGGCTG CTGGAGCATC TGGCCAGCCC AGAGCACAAG AAAGCAACCA ACAAATTCTG 360

GTGGGAGAAC AAAGCTGAGG TCCAGATGAA AGAGAAGTTT CTGGTCACTC CCCAGGATTA 420

TGCGCGATTC AAGAAATCCA TGGTGAAAGG TTTGGATTCC TATGAAGAAA AGGAGGATAA 480

AGTGATCAAG GAGATGGCAG CTCAGATCCG TGAGGTGGAG CAGAGCCGAC AGGAGGTGGT 540

TCGGTCTGTC TTAGAGCCTC AGGCAGTGCC AGACCCAGAA GAGGGCTCTT CAGCACCTAG 600

AAGCTGGAAA GGGATGAACA GCCAAGTAGC TTCCAGCTTA CAGCAGCCCT CAAATTTGGA 660

CCTGCCACCA GCTCCAGAGC TTGACTGGAT GGAGACAGGA CCATCTCTGA CATTCATTGG 720

CCATCAGGAT ATACCAGGAG TTGGTAACAT CCACTCAGGT GCCACACCTC CCTGGATGAT 780

CCAAGATGAA GAATACATTG CTGGGAACCA AGAAATAGGA CCATCCTATG AAGAATTTCT 840

TAAAGAAAAG GAAAAACAGA AGTTGAAAAA ACTCCCCCCA GACCGAGTTG GGGCCAACTT 900

TGATCACAGC TCCAGGACCA GTGCAGGCTG GCTGCCCTCT TTTGGGCCGC GTCTGGAATA 960

ATGGACGCCG CTGGCAGTCC AGACATCAAC TCCAAAACTG AAGCTGCAGC AATGAAGAAG 1020

CAGTCACATA CAGAAAAAAG CTAATCATGC TCTCTACCAA CTACCATGAG GCTAAAAGCC 1080

AAAGTCAACC AAACCCCTAT TATACCTTCC ACCCAAATTC TTTATCATTG TCTTTCTTAG 1140

GAAACAGACA TACTCATTCA TTTGATTTAA TAAAGTTTTA TTTTTCGGCC TTCGTGGCCT 1200

CGAATCAAGC TTATCGATAC CGTCGACCTC GAGGGGGGGC CGTACCCACT TTT 1253

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 417 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Ile Gly Val Thr Arg Trp Arg Arg Ser Arg Ile Val Asp Pro Arg Ala
1 5 10 15

Ala Xaa Asn Ser Ile Arg Ala His Glu Gly Pro Phe Phe Cys Gly Arg
20 25 30

Gly Thr Phe Thr Ala Ala Ser Thr Gln Arg Gln Leu Lys Glu Ala Phe
35 40 45

Glu Arg Leu Leu Pro Gln Val Glu Ala Ala Arg Lys Ala Ile Arg Ala
50 55 60

Ala Gln Val Glu Arg Tyr Val Pro Glu His Glu Arg Cys Cys Trp Cys
65 70 75 80

Leu Cys Cys Gly Cys Glu Val Arg Glu His Leu Ser His Gly Asn Leu
85 90 95

Thr Val Leu Tyr Gly Gly Leu Leu Glu His Leu Ala Ser Pro Glu His
100 105 110

Lys Lys Ala Thr Asn Lys Phe Trp Trp Glu Asn Lys Ala Glu Val Gln
115 120 125

Met Lys Glu Lys Phe Leu Val Thr Pro Gln Asp Tyr Ala Arg Phe Lys
130 135 140

Lys Ser Met Val Lys Gly Leu Asp Ser Tyr Glu Glu Lys Glu Asp Lys
145 150 155 160

Val Ile Lys Glu Met Ala Ala Gln Ile Arg Glu Val Glu Gln Ser Arg
165 170 175

Gln Glu Val Val Arg Ser Val Leu Glu Pro Gln Ala Val Pro Asp Pro
180 185 190

Glu Glu Gly Ser Ser Ala Pro Arg Ser Trp Lys Gly Met Asn Ser Gln
195 200 205

Val Ala Ser Ser Leu Gln Gln Pro Ser Asn Leu Asp Leu Pro Pro Ala
210 215 220

Pro Glu Leu Asp Trp Met Glu Thr Gly Pro Ser Leu Thr Phe Ile Gly
225 230 235 240

His Gln Asp Ile Pro Gly Val Gly Asn Ile His Ser Gly Ala Thr Pro
245 250 255

Pro Trp Met Ile Gln Asp Glu Glu Tyr Ile Ala Gly Asn Gln Glu Ile
260 265 270

Gly Pro Ser Tyr Glu Glu Phe Leu Lys Glu Lys Glu Lys Gln Lys Leu
275 280 285

Lys Lys Leu Pro Pro Asp Arg Val Gly Ala Asn Phe Asp His Ser Ser
290 295 300

Arg Thr Ser Ala Gly Trp Leu Pro Ser Phe Gly Pro Arg Leu Glu Xaa
305 310 315 320

Trp Thr Pro Leu Ala Val Gln Thr Ser Thr Pro Lys Leu Lys Leu Gln
325 330 335

Gln Xaa Arg Ser Ser His Ile Gln Lys Lys Ala Asn His Ala Leu Tyr
340 345 350

Gln Leu Pro Xaa Gly Xaa Lys Pro Lys Ser Thr Lys Pro Leu Leu Tyr
355 360 365

Leu Pro Pro Lys Phe Phe Ile Ile Val Phe Leu Arg Lys Gln Thr Tyr
370 375 380

Ser Phe Ile Xaa Phe Asn Lys Val Leu Phe Phe Gly Leu Arg Gly Leu
385 390 395 400

Glu Ser Ser Leu Ser Ile Pro Ser Thr Ser Arg Gly Gly Arg Thr His
405 410 415

Phe

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4596 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

AGCGGGGGGA CTGTGCCGTG TGGAACGTGT AGCTGTTGAA GGTGGACTCT GTTACCATTG 60

AGGATGTTTG GAGGATGAGT ATGTGTGGCA GAGGCACACA TAAACAGGCA GAGACCCTTT 120

GCCCCTGCCT TTCTCCCCCA ACCCAAGGCT GACCTGTGTT CTCCCAGGTC TGGGATTCTA 180

AGTGACCTGC TCTGTGTTTG GTCTCTCTCA GGATGAGCAC AAGCCTGGGA GATGGCAGTG 240

ATGGAAATGG CCTGCCCAGG TGCCCCTGGC TCAGCAGTGG GGCAGCAGAA GGAACTCCCC 300

AAGCCAAAGG AGAAGACGCC GCCACTGGGG AAGAAACAGA GCTCCGTCTA CAAGCTTGAG 360

GCCGTGGAGA AGAGCCCTGT GTTCTGCGGA AAGTGGGAGA TCCTGAATGA CGTGATTACC 420

AAGGGCACAG CCAAGGAAGG CTCCGAGGCA GGGCCAGCTG CCATCTCTAT CATCGCCCAG 480

GCTGAGTGTG AGAATAGCCA AGAGTTCAGC CCCACCTTTT CAGAACGCAT TTTCATCGCT 540

GGGTCCAAAC AGTACAGCCA GTCCGAGAGT CTTGATCAGA TCCCCAACAA TGTGGCCCAT 600

GCTACAGAGG GCAAAATGGC CCGTGTGTGT TGGAAGGGAA AGCGTCGCAG CAAAGCCCGG 660

AAGAAACGGA AGAAGAAGAG CTCAAAGTCC CTGGCTCATG CAGGAGTGGC CTTGGCCAAA 720

CCCCTCCCCA GGACCCCTGA GCAGGAGAGC TGCACCATCC CAGTGCAGGA GGATGAGTCT 780

CCACTCGGCG CCCCATATGT TAGAAACACC CCGCAGTTCA CCAAGCCTCT GAAGGAACCA 840

GGCCTTGGGC AACTCTGTTT TAAGCAGCTT GGCGAGGGCC TACGGCCGGC TCTGCCTCGA 900

TCAGAACTCC ACAAACTGAT CAGCCCCTTG CAATGTCTGA ACCACGTGTG GAAACTGCAC 960

CACCCCCAGG ACGGAGGCCC CCTGCCCCTG CCCACGCACC CCTTCCCCTA TAGCAGACTG 1020

CCTCATCCCT TCCCATTCCA CCCTCTCCAG CCCTGGAAAC CTCACCCTCT GGAGTCCTTC 1080

CTGGGCAAAC TGGCCTGTGT AGACAGCCAG AAACCCTTGC CTGACCCACA CCTGAGCAAA 1140

CTGGCCTGTG TAGACAGTCC AAAGCCCCTG CCTGGCCCAC ACCTGGAGCC CAGCTGCCTG 1200

TCTCGTGGTG CCCATGAGAA GTTTTCTGTG GAGGAATACC TAGTGCATGC TCTGCAAGGC 1260

AGCGTGAGCT CAAGCCAGGC CCACAGCCTG ACCAGCCTGG CCAAGACCTG GGCAGCACGG 1320

GGCTCCAGAT CCCGGGAGCC CAGCCCCAAA ACTGAGGACA ACGAGGGTGT CCTGCTCACT 1380

GAGAAACTCA AGCCAGTGGA TTATGAGTAC CGAGAAGAAG TCCACTGGGC CACGCACCAG 1440

CTCCGCCTGG GCAGAGGCTC CTTCGGAGAG GTGCACAGGA TGGAGGACAA GCAGACTGGC 1500

TTCCAGTGCG CTGTCAAAAA GGTGCGCCTG GAAGTATTTC GGGCAGAGGA GCTGATGGCA 1560

TGTGCAGGAT TGACCTCACC CAGAATTGTC CCTTTGTATG GAGCTGTGAG AGAAGGGCCT 1620

TGGGTCAACA TCTTCATGGA GCTGCTGGAA GGTGGCTCCC TGGGCCAGCT GGTCAAGGAG 1680

CAGGGCTGTC TCCCAGAGGA CCGGGCCCTG TACTACCTGG GCCAGGCCCT GGAGGGTCTG 1740

GAATACCTCC ACTCACGAAG GATTCTGCAT GGGGACGTCA AAGCTGACAA CGTGCTCCTG 1800

TCCAGCGATG GGAGCCACGC AGCCCTCTGT GACTTTGGCC ATGCTGTGTG TCTTCAACCT 1860

GATGGCCTGG GAAAGTCCTT GCTCACAGGG GACTACATCC CTGGCACAGA GACCCACATG 1920

GCTCCGGAGG TGGTGCTGGG CAGGAGCTGC GACGCCAAGG TGGATGTCTG GAGCAGCTGC 1980

TGTATGATGC TGCACATGCT CAACGGCTGC CACCCCTGGA CTCAGTTCTT CCGAGGGCCG 2040

CTCTGCCTCA AGATTGCCAG CGAGCCTCCG CCTGTGAGGG AGATCCCACC CTCCTGCGCC 2100

CCTCTCACAG CCCAGGCCAT CCAAGAGGGG CTGAGGAAAG AGCCCATCCA CCGCGTGTCT 2160

GCAGCGGAGC TGGGAGGGAA GGTGAACCGG GCACTACAGC AAGTGGGAGG TCTGAAGAGC 2220

CCTTGGAGGG GAGAATATAA AGAACCAAGA CATCCACCGC CAAATCAAGC CAATTACCAC 2280

CAGACCCTCC ATGCCCAGCC GAGAGAGCTT TCGCCAAGGG CCCCAGGGCC CCGGCCAGCT 2340

GAGGAGACAA CAGGCAGAGC CCCTAAGCTC CAGCCTCCTC TCCCACCAGA GCCCCCAGAG 2400

CCAAACAAGT CTCCTCCCTT GACTTTGAGC AAGGAGGAGT CTGGGATGTG GGAACCCTTA 2460

CCTCTGTCCT CCCTGGAGCC AGCCCCTGCC AGAAACCCCA GCTCACCAGA GCGGAAAGCA 2520

ACCGTCCCGG AGCAGGAACT GCAGCAGCTG GAAATAGAAT TATTCCTCAA CAGCCTGTCC 2580

CAGCCATTTT CTCTGGAGGA GCAGGAGCAA ATTCTCTCGT GCCTCAGCAT CGACAGCCTC 2640

TCCCTGTCGG ATGACAGTGA GAAGAACCCA TCAAAGGCCT CTCAAAGCTC GCGGGACACC 2700

CTGAGCTCAG GCGTACACTC CTGGAGCAGC CAGGCCGAGG CTCGAAGCTC CAGCTGGAAC 2760

ATGGTGCTGG CCCGGGGGCG GCCCACCGAC ACCCCAAGCT ATTTCAATGG TGTGAAAGTC 2820

CAAATACAGT CTCTTAATGG TGAACACCTG CACATCCGGG AGTTCCACCG GGTCAAAGTG 2880

GGAGACATCG CCACTGGCAT CAGCAGCCAG ATCCCAGCTG CAGCCTTCAG CTTGGTCACC 2940

AAAGACGGGC AGCCTGTTCG CTACGACATG GAGGTGCCAG ACTCGGGCAT CGACCTGCAG 3000

TGCACACTGG CCCCTGATGG CAGCTTCGCC TGGAGCTGGA GGGTCAAGCA TGGCCAGCTG 3060

GAGAACAGGC CCTAACCCTG CCCTCCACCG CCGGCTCCAC ACTGCCGGAA AGCAGCCTTC 3120

CTGCTCGGTG CACGATGCTG CCCTGAAAAC ACAGGCTCAG CCGTTCCCAG GGGATTGCCA 3180

GCCCCCCGGC TCACAGTGGG AACCAGGGCC TCGCAGCAGC AAGGTGGGGG CAAGCAGAAT 3240

GCCTCCCAGG ATTTCACACC TGAGCCCTGC CCCACCCTGC TGAAAAAACA TCCGCCACGT 3300

GAAGAGACAG AAGGAGGATG GCAGGAGTTA CCTGGGGAAA CAAAACAGGG ATCTTTTTCT 3360

GCCCCTGCTC CAGTCGAGTT GGCCTGACCC GCTTGGATCA GTGACCATTT GTTGGCAGAC 3420

AGGGGAGAGC AGCTTCCAGC CTGGGTCAGA AGGGGTGGGC GAGCCCTTCG GCCCCTCACC 3480

CTCCAGGCTG CTGTGAGAGT GTCAAGTGTG TAAGGGCCCA AACTCAGGTT CAGTGCAGAA 3540

CCAGGTCAGC AGGTATGCCC GCCCGTAGGT TAAGGGGGCC CTCTAAACCC CTTGCCTGGC 3600

CTCACCTGGC CAGCTCACCC CTTTTGGGTG TAGGGGAAAA GAATGCCTGA CCCTGGGAAG 3660

GCTCCCTGGT AGAATACACC ACACTTTTCA GGTTGTTGCA ACACAGGTCC TGAGTTGACC 3720

TCTGGTTCAG CCAAGGACCA AAGAAGGTGT GTAAGTGAAG TGGTTCTCAG TCCCCAGACA 3780

TGTGCCCCTT TGCTGCTGGC TACCACTCTT CCCCAGAGCA GCAGGCCCCG AGCCCCTTCA 3840

GGCCCAGCAC TGCCCCAGAC TCGCTGGCAC TCAGTTCCCT CATCTGTAAA GGTGAAGGGT 3900

GATGCAGGAT ATGCCTGACA GGAACAGTCT GTGGATGGAC ATGATCAGTG CTAAGGAAAG 3960

CAGCAGAGAG AGACGTCCGG CGCCCCAGCC CCACTATCAG TGTCCAGCGT GCTGGTTCCC 4020

CAGAGCACAG CTCAGCATCA CACTGACACT CACCCTGCCC TGCCCCTGGC CAGAGGGTAC 4080

TGCCGACGGC ACTTTGCACT CTGATGACCT CAAAGCACTT TCATGGCTGC CCTCTGGCAG 4140

GGCAGGGCAG GGCAGTGACA CTGTAGGAGC ATAGCAAGCC AGGAGATGGG GTGAAGGGAC 4200

ACAGTCTTGA GCTGTCCACA TGCATGTGAC TCCTCAAACC TCTTCCAGAT TTCTCTAAGA 4260

ATAGCACCCC CTTCCCCATT GCCCCAGCTT AGCCTCTTCT CCCAGGGGAG CTACTCAGGA 4320

CTCACGTAGC ATTAAATCAG CTGTGAATCG TCAGGGGGTG TCTGCTAGCC TCAACCTCCT 4380

GGGGCAGGGG ACGCCGAGAC TCCGTGGGAG AAGCTCATTC CCACATCTTG CCAAGACAGC 4440

CTTTGTCCAG CTGTCCACAT TGAGTCAGAC TGCTCCCGGG GAGAGAGCCC CGGCCCCCAG 4500

CACATAAAGA ACTGCAGCCT TGGTACTGCA GAGTCTGGGT TGTAGAGAAC TCTTTGTAAG 4560

CAATAAAGTT TGGGGTGATG ACAAATGTTA AAAAAA 4596

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 947 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

```
Met Ala Val Met Glu Met Ala Cys Pro Gly Ala Pro Gly Ser Ala Val
1               5                  10                  15

Gly Gln Gln Lys Glu Leu Pro Lys Pro Lys Glu Lys Thr Pro Pro Leu
            20                  25                  30

Gly Lys Lys Gln Ser Ser Val Tyr Lys Leu Glu Ala Val Glu Lys Ser
        35                  40                  45

Pro Val Phe Cys Gly Lys Trp Glu Ile Leu Asn Asp Val Ile Thr Lys
    50                  55                  60

Gly Thr Ala Lys Glu Gly Ser Glu Ala Gly Pro Ala Ala Ile Ser Ile
65                  70                  75                  80

Ile Ala Gln Ala Glu Cys Glu Asn Ser Gln Glu Phe Ser Pro Thr Phe
                85                  90                  95
```

Ser Glu Arg Ile Phe Ile Ala Gly Ser Lys Gln Tyr Ser Gln Ser Glu
100 105 110

Ser Leu Asp Gln Ile Pro Asn Asn Val Ala His Ala Thr Glu Gly Lys
115 120 125

Met Ala Arg Val Cys Trp Lys Gly Lys Arg Arg Ser Lys Ala Arg Lys
130 135 140

Lys Arg Lys Lys Lys Ser Ser Lys Ser Leu Ala His Ala Gly Val Ala
145 150 155 160

Leu Ala Lys Pro Leu Pro Arg Thr Pro Glu Gln Glu Ser Cys Thr Ile
165 170 175

Pro Val Gln Glu Asp Glu Ser Pro Leu Gly Ala Pro Tyr Val Arg Asn
180 185 190

Thr Pro Gln Phe Thr Lys Pro Leu Lys Glu Pro Gly Leu Gly Gln Leu
195 200 205

Cys Phe Lys Gln Leu Gly Glu Gly Leu Arg Pro Ala Leu Pro Arg Ser
210 215 220

Glu Leu His Lys Leu Ile Ser Pro Leu Gln Cys Leu Asn His Val Trp
225 230 235 240

Lys Leu His His Pro Gln Asp Gly Gly Pro Leu Pro Leu Pro Thr His
245 250 255

Pro Phe Pro Tyr Ser Arg Leu Pro His Pro Phe Pro Phe His Pro Leu
260 265 270

Gln Pro Trp Lys Pro His Pro Leu Glu Ser Phe Leu Gly Lys Leu Ala
275 280 285

Cys Val Asp Ser Gln Lys Pro Leu Pro Asp Pro His Leu Ser Lys Leu
290 295 300

Ala Cys Val Asp Ser Pro Lys Pro Leu Pro Gly Pro His Leu Glu Pro
305 310 315 320

Ser Cys Leu Ser Arg Gly Ala His Glu Lys Phe Ser Val Glu Glu Tyr
325 330 335

Leu Val His Ala Leu Gln Gly Ser Val Ser Ser Ser Gln Ala His Ser
340 345 350

Leu Thr Ser Leu Ala Lys Thr Trp Ala Ala Arg Gly Ser Arg Ser Arg
355 360 365

Glu Pro Ser Pro Lys Thr Glu Asp Asn Glu Gly Val Leu Leu Thr Glu
370 375 380

Lys Leu Lys Pro Val Asp Tyr Glu Tyr Arg Glu Glu Val His Trp Ala
385 390 395 400

Thr His Gln Leu Arg Leu Gly Arg Gly Ser Phe Gly Glu Val His Arg
405 410 415

Met Glu Asp Lys Gln Thr Gly Phe Gln Cys Ala Val Lys Lys Val Arg
420 425 430

Leu Glu Val Phe Arg Ala Glu Glu Leu Met Ala Cys Ala Gly Leu Thr
435 440 445

Ser Pro Arg Ile Val Pro Leu Tyr Gly Ala Val Arg Glu Gly Pro Trp
450 455 460

Val Asn Ile Phe Met Glu Leu Leu Glu Gly Gly Ser Leu Gly Gln Leu
465 470 475 480

Val Lys Glu Gln Gly Cys Leu Pro Glu Asp Arg Ala Leu Tyr Tyr Leu
485 490 495

Gly Gln Ala Leu Glu Gly Leu Glu Tyr Leu His Ser Arg Arg Ile Leu
500 505 510

His Gly Asp Val Lys Ala Asp Asn Val Leu Leu Ser Ser Asp Gly Ser
515 520 525

His Ala Ala Leu Cys Asp Phe Gly His Ala Val Cys Leu Gln Pro Asp
530 535 540

Gly Leu Gly Lys Ser Leu Leu Thr Gly Asp Tyr Ile Pro Gly Thr Glu
545 550 555 560

Thr His Met Ala Pro Glu Val Val Leu Gly Arg Ser Cys Asp Ala Lys
565 570 575

Val Asp Val Trp Ser Ser Cys Cys Met Met Leu His Met Leu Asn Gly
580 585 590

Cys His Pro Trp Thr Gln Phe Phe Arg Gly Pro Leu Cys Leu Lys Ile
595 600 605

Ala Ser Glu Pro Pro Pro Val Arg Glu Ile Pro Pro Ser Cys Ala Pro
610 615 620

Leu Thr Ala Gln Ala Ile Gln Glu Gly Leu Arg Lys Glu Pro Ile His
625 630 635 640

Arg Val Ser Ala Ala Glu Leu Gly Gly Lys Val Asn Arg Ala Leu Gln
645 650 655

Gln Val Gly Gly Leu Lys Ser Pro Trp Arg Gly Glu Tyr Lys Glu Pro
660 665 670

Arg His Pro Pro Pro Asn Gln Ala Asn Tyr His Gln Thr Leu His Ala
675 680 685

Gln Pro Arg Glu Leu Ser Pro Arg Ala Pro Gly Pro Arg Pro Ala Glu
690 695 700

Glu Thr Thr Gly Arg Ala Pro Lys Leu Gln Pro Pro Leu Pro Pro Glu
705 710 715 720

Pro Pro Glu Pro Asn Lys Ser Pro Pro Leu Thr Leu Ser Lys Glu Glu
725 730 735

Ser Gly Met Trp Glu Pro Leu Pro Leu Ser Ser Leu Glu Pro Ala Pro

740 745 750

Ala Arg Asn Pro Ser Ser Pro Glu Arg Lys Ala Thr Val Pro Glu Gln
755 760 765

Glu Leu Gln Gln Leu Glu Ile Glu Leu Phe Leu Asn Ser Leu Ser Gln
770 775 780

Pro Phe Ser Leu Glu Glu Gln Glu Gln Ile Leu Ser Cys Leu Ser Ile
785 790 795 800

Asp Ser Leu Ser Leu Ser Asp Asp Ser Glu Lys Asn Pro Ser Lys Ala
805 810 815

Ser Gln Ser Ser Arg Asp Thr Leu Ser Ser Gly Val His Ser Trp Ser
820 825 830

Ser Gln Ala Glu Ala Arg Ser Ser Ser Trp Asn Met Val Leu Ala Arg
835 840 845

Gly Arg Pro Thr Asp Thr Pro Ser Tyr Phe Asn Gly Val Lys Val Gln
850 855 860

Ile Gln Ser Leu Asn Gly Glu His Leu His Ile Arg Glu Phe His Arg
865 870 875 880

Val Lys Val Gly Asp Ile Ala Thr Gly Ile Ser Ser Gln Ile Pro Ala
885 890 895

Ala Ala Phe Ser Leu Val Thr Lys Asp Gly Gln Pro Val Arg Tyr Asp
900 905 910

Met Glu Val Pro Asp Ser Gly Ile Asp Leu Gln Cys Thr Leu Ala Pro
915 920 925

Asp Gly Ser Phe Ala Trp Ser Trp Arg Val Lys His Gly Gln Leu Glu
930 935 940

Asn Arg Pro
945

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide PCR primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

CAGGATCCTC ATGGCTGCAG CTAGCGTGAC 30

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide PCR primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GGTCGACTTA GAGCCCTGTC AGGTCCACAA TG 32

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide probe"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GATGCCATTG GGGATTTCCT CTTT 24

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide probe"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

CAGTAAAGAG GAAATCCCCA ATGG 24

Combined Declaration for Patent Application and Power of Attorney

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name; and that I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled
MODULATORS OF TNF RECEPTOR ASSOCIATED FACTOR (TRAF), THEIR PREPARATION AND USE

the specification of which (check one)

[ ] is attached hereto;
[ ] was filed in the United States under 35 U.S.C. §111 on _____, as USSN _____*; or
[XX] was/will be filed in the U.S. under 35 U.S.C. §371 by entry into the U.S. national stage of an international (PCT) application, PCT/IL97/00117 ; filed 01 April 1997 , entry requested on 02 October 1998 *; national stage application received USSN _____*; §371/§102(e) date _____* (*if known),

and was amended on 02 Octobe 1998 (if applicable).

(include dates of amendments under PCT Art. 19 and 34 if PCT)

I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above; and I acknowledge the duty to disclose to the Patent and Trademark Office (PTO) all information known by me to be material to patentability as defined in 37 C.F.R. § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §§ 119, 365 of any prior foreign application(s) for patent or inventor's certificate, or prior PCT application(s) designating a country other than the U.S., listed below with the "Yes" box checked and have also identified below any such application having a filing date before that of the application on which priority is claimed:

117800 (Number)	Israel (Country)	02 April 1996 (Day Month Year Filed)	[X] YES	[ ] NO
119133 (Number)	Israel (Country)	26 August 1996 (Day Month Year Filed)	[X] YES	[ ] NO

I hereby claim the benefit under 35 U.S.C. § 120 of any prior U.S. non-provisional Application(s) or prior PCT Application(s) designating the U.S. listed below, or under § 119(e) of any prior U.S. provisional applications listed below, and, insofar as the subject matter of each of the claims of this application is not disclosed in such U.S. or PCT application in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the PTO all information as defined in 37 C.F.R. §1.56(a) which occurred between the filing date of the prior application and the national filing date of this application:

(Application Serial No.)	(Day Month Year Filed)	(Status: patented, pending, abandoned)
(Application Serial No.)	(Day Month Year Filed)	(Status: patented, pending, abandoned)
(Application Serial No.)	(Day Month Year Filed)	(Status: patented, pending, abandoned)

I hereby appoint the following attorneys, with full power of substitution, association, and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

SHERIDAN NEIMARK, REG. NO. 20,520 - ROGER L. BROWDY, REG. NO. 25,618 - ANNE M. KORNBAU, REG. NO. 25,884
NORMAN J. LATKER, REG. NO. 19,963 - IVER P. COOPER, REG. NO. 28,005 - ALLEN C. YUN, REG. NO. 37,971*
NICK S. BROMER, REG. NO. 33,478 - * Patent Agent

ADDRESS ALL CORRESPONDENCE TO	DIRECT ALL TELEPHONE CALLS TO:
BROWDY AND NEIMARK, P.L.L.C. 419 Seventh Street, N.W. Washington, D.C. 20004	BROWDY AND NEIMARK (202) 628-5197

The undersigned hereby authorizes the U.S. Attorneys or Agents named herein to accept and follow instructions from INTER-LAB LTD. as to any action to be taken in the U.S. Patent and Trademark Office regarding this application without direct communication between the U.S. Attorney or Agent and the undersigned. In the event of a change of the persons from whom instructions may be taken, the U.S. Attorneys or Agents named herein will be so notified by the undersigned.

Atty.Docket: WALLACH=21

Title: MODULATORS OF TNF RECEPTOR ASSOCIATED FACTOR (TRAF), THEIR...

U.S. Application filed _____, Serial No. _____

PCT Application filed 01 April 1997, Serial No. PCT/IL97/00117

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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FULL NAME OF FIRST INVENTOR	INVENTOR'S SIGNATURE	DATE
David WALLACH	x	15/10/98
RESIDENCE: Rehovot, Israel ILX	CITIZENSHIP: Israel	
POST OFFICE ADDRESS: 24 Borochov Street, 76406 Rehovot, Israel		

FULL NAME OF SECOND JOINT INVENTOR	INVENTOR'S SIGNATURE	DATE
2 Nikolai MALININ	x	15 Nov. 98
RESIDENCE: Rehovot, Israel ILX	CITIZENSHIP: Russian	
POST OFFICE ADDRESS: Weizmann Institute of Science, Beit Clore, 76100 Rehovot, Israel		

FULL NAME OF THIRD JOINT INVENTOR	INVENTOR'S SIGNATURE	DATE
3 Mark BOLDIN	x	3/11/98
RESIDENCE: Rehovot, Israel ILX	CITIZENSHIP: Russian	
POST OFFICE ADDRESS: Weizmann Institute of Science, Beit Clore, 76100 Rehovot, Israel		

FULL NAME OF FOURTH JOINT INVENTOR	INVENTOR'S SIGNATURE	DATE
4 Andrei KOVALENKO	x	15/11/98
RESIDENCE: Rehovot, Israel ILX	CITIZENSHIP: Russian	
POST OFFICE ADDRESS: Weizmann Institute of Science, Beit Clore, 76100 Rehovot, Israel		

FULL NAME OF FIFTH JOINT INVENTOR	INVENTOR'S SIGNATURE	DATE
5 Igor METT	x	20/12/98
RESIDENCE: Rehovot, Israel ILX	CITIZENSHIP: Israel	
POST OFFICE ADDRESS: 60 Levin Epstein Street, 76462 Rehovot, Israel		

FULL NAME OF SIXTH JOINT INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		

FULL NAME OF SEVENTH JOINT INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		

ALL INVENTORS MUST REVIEW APPLICATION AND DECLARATION BEFORE SIGNING. ALL ALTERATIONS MUST BE INITIALED AND DATED BY ALL INVENTORS PRIOR TO EXECUTION. NO ALTERATIONS CAN BE MADE AFTER THE DECLARATION IS SIGNED. ALL PAGES OF DECLARATION MUST BE SEEN BY ALL INVENTORS.